

Review

Physical Pretreatments of Lignocellulosic Biomass for Fermentable Sugar Production

Damázio Borba Sant'Ana Júnior, Maikon Kelbert , Pedro Henrique Hermes de Araújo 
and Cristiano José de Andrade * 

Department of Chemical and Food Engineering, Federal University of Santa Catarina, Florianopolis 88040-900, SC, Brazil; damaziojunior@hotmail.com (D.B.S.J.); maikonkelbert@gmail.com (M.K.); pedro.h.araujo@ufsc.br (P.H.H.d.A.)

* Correspondence: eng.crisja@gmail.com

Abstract: Physical pretreatments play a crucial role in reducing the recalcitrance of lignocellulosic biomass, facilitating its conversion into fermentable sugars for bioenergy and chemical applications. This study critically reviews physical pretreatment approaches, including mechanical comminution, irradiation (ultrasound, microwave, gamma rays, and electron beam), extrusion, and pulsed electric field. The discussion covers the mechanisms of action, operational parameters, energy efficiency, scalability challenges, and associated costs. Methods such as ultrasound and microwave induce structural changes that enhance enzymatic accessibility, while extrusion combines thermal and mechanical forces to optimize hydrolysis. Mechanical comminution is most effective during short periods and when combined with other techniques to overcome limitations such as high energy consumption. Innovative approaches, such as pulsed electric fields, show significant potential but face challenges in large-scale implementation. This study provides technical and strategic insights into developing more effective physical pretreatments aligned with economic feasibility and industrial sustainability.

Keywords: lignocellulosic biomass; physical pretreatment; enzymatic hydrolysis; mechanical comminution; irradiation; ultrasound; microwave; gamma rays; electron beam; extrusion; pulsed electric field



Academic Editor: Daily
Rodríguez-Padrón

Received: 27 December 2024

Revised: 18 March 2025

Accepted: 1 April 2025

Published: 14 April 2025

Citation: Sant'Ana Júnior, D.B.; Kelbert, M.; Hermes de Araújo, P.H.; de Andrade, C.J. Physical Pretreatments of Lignocellulosic Biomass for Fermentable Sugar Production. *Sustain. Chem.* **2025**, *6*, 13. <https://doi.org/10.3390/suschem6020013>

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Fossil resources are the primary energy source in the world, resulting in significant industrial advances that have promoted a better quality of life for society [1]. Although using oil and its derivatives has brought several advantages, the exhaustion of fossil fuel reserves and the search for new sustainable approaches have been promoted to avoid their environmental impacts [2–4].

Lignocellulosic biomass is a sustainable option, consisting of plant sources, agro-industrial residues, and organic material from its processing [5]. It is mainly composed of cellulose, hemicellulose, and lignin [6]. However, other components such as chlorophyll, proteins, resins, pectin, ash, terpenoids, and other extractives can be part of its composition [7]. Moreover, the biological conversion of lignocellulosic biomass can be performed effectively to generate economically viable bio-based products by processing it in a biorefinery [8–10].

In biorefineries, saccharification is a crucial process that converts lignocellulosic material into fermentable sugars. These sugars can be used as feedstock for producing biofuels,

biochemicals, biomaterials, and other products [11]. This step involves the enzymatic hydrolysis of the glucan and xylan portions, involving hydrolytic enzymes such as cellulases and hemicellulases [12]. In addition, an efficient pretreatment can enhance the conversion process by breaking down the lignin structure and disrupting the crystalline structure of cellulose, thereby facilitating enzyme access to cellulose during hydrolysis [13].

In this context, pretreatment is a fundamental step in preparing lignocellulosic biomass for further processing into value-added chemicals [14]. It is necessary to break the recalcitrant structure, leaving the cellulose and hemicellulose more accessible to enzymes and chemicals [15]. It also facilitates further biomass processing by effectively removing lignin, degrading hemicellulose, reducing cellulose crystallinity, and increasing surface porosity [16]. The pretreatment of lignocellulosic biomass is a fundamental step in biorefinery and is essential for obtaining a high product yield [17]. It is estimated that pretreatment can account for up to 40% of the total costs of the overall biorefinery process [18]. Pretreatment processes can generally be divided into four categories: physical, chemical, biological, and combined techniques [19]. Each of these pretreatments has different advantages and disadvantages that make them more or less suitable for various types of biomass and conversion processes [20].

Chemical pretreatments are more effective in dissolving, hydrolyzing, or oxidizing biomass components, making them more accessible for conversion, but can generate toxic by-products and impair the yield of subsequent processes [21,22]. Biological pretreatments are generally more specific to biomass types and conversion processes and can be performed under milder conditions [13,23]. However, they may take longer to degrade the biomass [24]. Physical pretreatments, on the other hand, are generally more effective in reducing particle size and increasing particle surface area, as well as damaging the recalcitrant structure of the lignocellulosic biomass. They do not produce inhibitor products and are faster processes but may require specific equipment and conditions that can increase the cost of the process [13,15].

This work critically discusses the effects of physical pretreatments on lignocellulosic biomass, covering aspects such as the main pretreatment mechanisms, variation in lignocellulosic biomass, operational parameters, production of fermentable sugars, energy consumption, costs, and scalability. Lastly, the challenges and future perspectives are discussed, highlighting the factors affecting how to conduct the pretreatment.

2. Lignocellulosic Biomass

Lignocellulosic biomass is a promising renewable resource for producing biofuels and biochemicals [24]. This biomass consists mainly of plant residues, agricultural by-products, and waste materials [25]. Structurally, it comprises three primary components: cellulose, hemicellulose, and lignin, which vary in proportion depending on the biomass source (Figure 1) [12,26].

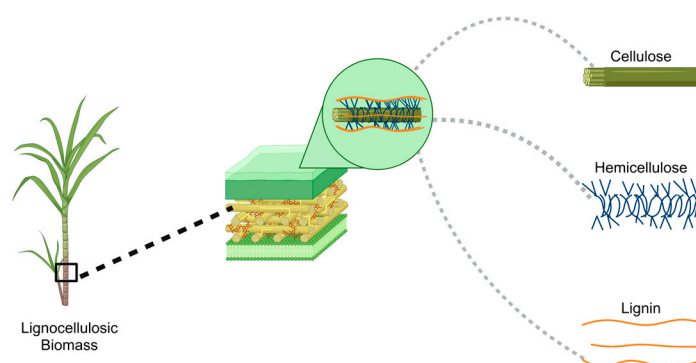


Figure 1. Schematic representation of the structural configuration of lignocellulosic biomass.

Typically, cellulose accounts for 35% to 50% of the biomass, hemicellulose for 20% to 35%, and lignin for 5% to 30% [27–29]. Their structure makes lignocellulosic biomass naturally recalcitrant, hindering degradation [28,30]. Integrating lignocellulosic biomass into the circular economy can reduce reliance on non-renewable resources and minimize waste through sustainable conversion into bio-based products [31].

Cellulose, a glucose polymer, has a highly crystalline structure that resists enzymatic breakdown [13]. Composed of β -1,4 glycosidic bonds, it hinders direct conversion into fermentable sugars, necessitating pretreatment to disrupt this crystalline form [32]. Hemicellulose, in contrast, is a heterogeneous polymer composed of sugars such as xylose, mannose, and arabinose. Being amorphous and less polymerized, it is easier to remove during pretreatment [18,31]. Lignin, an aromatic and highly recalcitrant macromolecule, hinders enzymatic access to cellulose and hemicellulose, requiring depolymerization to enhance conversion efficiency [18].

For pretreatment selection, woody and non-woody biomasses differ in resistance to degradation and pretreatment effectiveness. Woody biomass, which includes hardwoods and softwoods, is more recalcitrant due to its higher lignin content [13]. Examples include eucalyptus and pine, which are commonly used in industrial bioconversion processes. On the other hand, non-woody biomass consists of agricultural residues like wheat straw, corn cobs, sugarcane bagasse, corn husks, soybean hulls, and grasses [14].

This research builds upon classifications provided by Yousuf et al. [14], which categorize all biomass types as either woody or non-woody, and Wang et al. [33], which focuses on lignin content, thereby including biomass with varying lignin compositions. Furthermore, this study considered residues from Brazil's three significant crops—soybeans, corn, and sugarcane—based on IBGE data [34]. Understanding the composition and classification of lignocellulosic biomass is pivotal in selecting effective pretreatment strategies, ensuring optimized conversion efficiency and economic viability.

3. Physical Pretreatment

Physical pretreatment uses mechanical forces, pressure, or temperature to induce structural changes in lignocellulosic biomass [14,35]. It reduces particle size, degree of polymerization, and crystallinity index while increasing specific surface area [36]. Additionally, it helps minimize inhibitor production for subsequent reactions [10,14]. Physical pretreatments are categorized into mechanical comminution, irradiation (ultrasound, microwave, gamma rays), electron beam, extrusion, and pulsed electric field (Figure 2) [14,19].

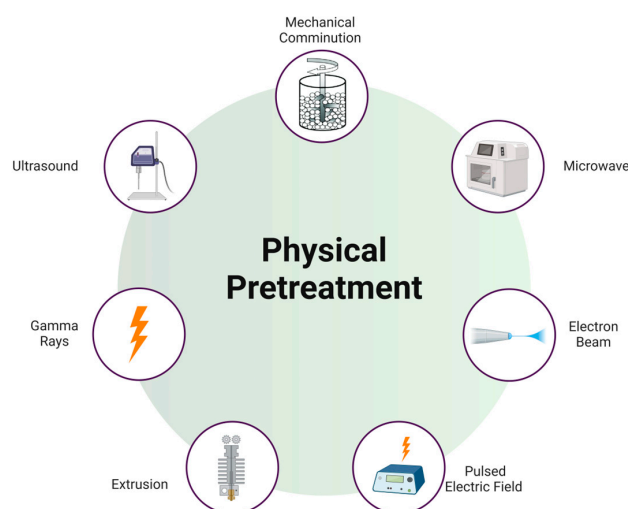


Figure 2. Schematic representation of physical pretreatments categorized as mechanical comminution, irradiation (ultrasound, microwave, gamma rays), electron beam, extrusion, and pulsed electric field.

3.1. Mechanical Comminution Pretreatment

Mechanical comminution involves the physical breakdown of the material into smaller particles [37]. This process is usually achieved through mechanical force, such as chipping, grinding, and milling, with the choice of milling method depending on the desired final particle size. Several types of milling equipment are available, including ball mills, hammer mills, knife mills, vibro mills, two-roll mills, colloid mills, wet-disk mills, and attrition mills [14,23,38].

Mechanical comminution improves the biodegradability of lignocellulosic biomass by decreasing the degree of polymerization, reducing crystallinity, and increasing the accessible surface area [16]. Nevertheless, it can produce heat that leads to the thermal degradation of biomass components, requiring careful regulation of duration and intensity [39].

In the biomass studied, mechanical comminution has proven essential in improving enzymatic accessibility and the efficiency of lignocellulosic biomass conversion processes. It is often considered a preliminary step before more complex pretreatments, such as enzymatic hydrolysis or chemical treatments. However, few studies analyze the isolated effect of this method. Refs. [40–43] demonstrate that particle size reduction alone significantly improves enzymatic accessibility and biomass conversion efficiency. Table 1 presents these studies along with the main parameters and results obtained:

Table 1. Studies on the use of mechanical comminution to produce fermentable sugars.

Biomass	Operation Type	Specific Parameters	Saccharification Parameters	Main Results	Reference
Sugarcane bagasse	Ball Milling	Initial size: 1000 μm , 250 rpm, 20 mm balls, up to 240 min	5% (<i>w/v</i>) pretreated bagasse, 5 FPU/g substrate Acremonium cellulase, 20 U/g xylanase (Optimash BG), 50 mM acetate buffer (pH 5.0), 45 $^{\circ}\text{C}$, 72 h	Glucose yield: 89.2%, Xylose yield: 77.2%; Significant particle size reduction (not specified)	[40]
Corn stover	Grinding (mill)	Initial size: 425–710 μm	15 FPU/g glucan Spezyme CP, 65 IU/g glucan Novozyme 188; 0.5 M acetate buffer (pH 4.8), 50 $^{\circ}\text{C}$, 180 rpm	Final particle size: 53–75 μm ; Glucose yield: 20.8%	[41]
Sugarcane bagasse and straw	Ball Milling	Initial size: 2000 μm , 400 rpm, 15 mm balls, 30–120 min	15 FPU/g biomass, 5% substrate concentration, pH 5, 45 $^{\circ}\text{C}$, 72 h	Glucose yield: 78.7% (bagasse, 60 min), 77.6% (straw, 90 min)	[42]
Lodgepole pine	Disk Milling	Initial size: 760 to 1520 μm	15 FPU/g Acremonium cellulase, 0.02 mL Optimash BG, 5 IU Novozyme 188/g of dried biomass; 50 mM acetate buffer (pH 5.0); 45 $^{\circ}\text{C}$; 48 h	Final particle size: 760 to 1520 μm ; Cellulose-to-glucose conversion: 80%	[43]
Eucalyptus	Ball Milling	Initial size: 420 μm and 150 μm , 400 rpm, 15 mm balls, 10–120 min	1–40 FPU Acremonium cellulase, 0.02 mL Optimash BG, 5 IU Novozyme 188/g dry biomass; 50 mM acetate buffer (pH 5.0); 45 $^{\circ}\text{C}$; 72 h	Glucose yield: 46.8% (20 min), 89.7% (120 min); Xylose yield: 34.3% (20 min), 72.5% (120 min); Total sugar yield: 74% at 200 g/L biomass	[44]

Particle size before and after pretreatment is an essential factor in mechanical comminution. Buaban et al. [40] employed ball milling as a pretreatment method for sugarcane bagasse, with an initial particle size of 1000 μm . Similarly, Inoue et al. [44] used a ball mill to process eucalyptus wood, starting with particles of 35 mesh (420 μm) and 100 mesh (150 μm). Sant’Ana da Silva et al. [41] utilized ball milling for sugarcane bagasse and straw,

starting with 2000 μm . These studies reported a considerable reduction in particle size but did not provide final values. In contrast, Zeng et al. [42] showed that corn stover was reduced from 425–710 μm to 53–75 μm after ball milling. Zhu et al. [43] also reported that particle size ranged from 0.76 mm to 1.52 mm after disk milling lodgepole pine wood chips.

Milling speed plays a crucial role in the efficiency of mechanical pretreatment. Several studies report the use of ball milling at speeds of 400 rpm. Inoue et al. [44] observed that, by milling eucalyptus wood at 400 rpm for 120 min, the glucose saccharification yield reached 89.7%. Sant'Ana da Silva et al. [41] used the same speed when treating sugarcane bagasse and straw, achieving glucose yields of 78.7% and 77.6% and xylose yields of 72.1% and 56.8%, respectively. On the other hand, Buaban et al. [40] used a lower speed of 250 rpm when milling sugarcane bagasse, which required a longer time to achieve significant particle size reduction.

Zhu et al. [43] did not mention the rotation speed for disk milling, as this equipment operates differently from ball milling. However, even without the exact speed specification, the particle size range after treatment was well documented, varying from 0.76 mm to 1.52 mm. Mechanical comminution effectively reduces particle size, improving surface area and accessibility for enzymatic hydrolysis. However, the best results were obtained during long processing periods (over 60 min) to achieve significant modifications. These prolonged durations are necessary due to the recalcitrance of the biomass. The lignin content also plays a role, as lignin imparts rigidity to the particles, requiring either greater applied force or more extended milling periods, which increases energy consumption and costs—an unfavorable factor for industrial applications.

Although mechanical comminution improves saccharification yields by facilitating enzyme access, it is insufficient as a standalone pretreatment. This limitation arises from its inability to significantly disrupt the lignocellulosic matrix, including physical barriers and chemical bonds that inhibit enzymatic activity. Consequently, this step is often considered preliminary and is commonly combined with other methods to maximize sugar recovery. This highlights the importance of considering the specific characteristics of the biomass, such as lignin content and particle rigidity, when optimizing pretreatment strategies.

3.2. Irradiation Pretreatment

The irradiation pretreatment methods for lignocellulosic biomass encompass ultrasound, microwave, gamma rays, and electron beams [14]. The effect of irradiation on biomass and the process mechanisms vary depending on the method applied [45]. These methods involve electron beams generated by an electron accelerator or gamma rays from radioisotopes such as cobalt-60 to degrade the cellulosic structure [19,46].

In these processes, high-energy radiation is produced, changing the properties of lignocellulosic biomass by decreasing crystallinity and the degree of polymerization, increasing specific surface area, and causing hydrolysis of hemicellulose and depolymerization of part of lignin [47].

3.2.1. Ultrasound

The ultrasound pretreatment process uses supersonic waves with frequencies greater than 20 kHz, which exceed human hearing limits (20 Hz–20 kHz) [48]. These ultrasonic waves generate pressure variations within a solution, amplifying mechanoacoustic and sonochemical processes [49].

The mechanoacoustic effect is based on cavitation induced by ultrasound radiation [49]. In this process, ultrasound waves pass through low-pressure regions, forming small gas or vapor bubbles that expand until they reach a critical threshold, collapsing and triggering cavitation [50]. This implosion releases energy, generating temperatures of 2000 to 5000 K

and pressures up to 1800 atm. These conditions create shear forces that break the lignocellulosic biomass structure, improving the extraction efficiency of cellulose, hemicellulose, and lignin [51,52].

The sonochemical effect enhances chemical reactions and directs specific pathways [53]. Typically used in radical-driven processes, ultrasound accelerates reactions at lower temperatures while reducing the required chemicals [47,48]. In aqueous solutions, it breaks the oxygen–hydrogen (O–H) bond, generating hydroxyl and hydrogen radicals [47]. These radicals undergo further reactions, yielding hydrogen peroxide, hydrogen gas, oxygen gas, or recombining to form water. Additional reactions occur in the gaseous or aqueous phase, producing more radicals or oxidizing species [47]. The literature presents several studies investigating the use of ultrasound as a pretreatment for fermentable sugar production. These studies are detailed in Table 2, including the respective operating conditions.

Table 2. Studies on ultrasound pretreatment for biomass.

Biomass	Pretreatment Parameters	Main Results	Reference
<i>Eucalyptus urophylla</i> × <i>Eucalyptus grandis</i>	Power: 10–500 W; Frequency: 20–25 kHz; Time: 1, 3, and 6 h	Drying rate increased: 5% (1 h), 13% (3 h), 11% (6 h) above 24% moisture; 25% (1 h), 28% (3 h), 23% (6 h) below 24%. Improved permeability. Reduced wood collapse.	[54]
<i>Eucalyptus grandis</i> × <i>Eucalyptus urophylla</i>	Frequency: 28 kHz; Time: 0.5, 1, and 1.5 h	Caustic soda solution pretreatment increased reaction rates and reduced thermal decomposition temperatures (309–400 °C). Gas products (CO, H ₂ O, CO ₂ , CH ₄ , CH ₃ COOH) released more at 361 °C than 308 °C.	[55]
<i>Eucalyptus grandis</i> × <i>Eucalyptus urophylla</i>	Frequency: 28 kHz; Time: 0.5, 1, and 1.5 h; Temperature: 50 °C	Increased cellulose crystallinity from 31.8% (control) to 35.5% (alkaline pretreatment). Hemicellulose and lignin removal enhanced solvent exposure.	[56]
<i>Pinus sylvestris</i>	Frequency: 16.8–9.2 kHz; Time: 25 min	Glucose yield in enzymatic hydrolysis increased 2-fold (35.5 g/L, 61% theoretical). Ethanol yield: 3.11% (v/v) after fermentation.	[57]
Sugarcane Bagasse	Power: 40 W; Frequency: 20 kHz; Time: 20 min	Reducing sugar yield: 276.8 mg/g biomass (1.12× DES alone, 1.58× ultrasound alone). Crystallinity index decreased from 64.87% to 52.78%.	[58]
Sugarcane Straw	Frequency: 40 kHz; Time: 30 min	Cellulose conversion: Hydrothermal + NaOH: 86.74%; Acid + NaOH: 84.29%. Ultrasound was the least effective for lignin and hemicellulose removal.	[59]
Sugarcane Straw	Power: 180 and 800 W; Frequency: 19 kHz; Time: 30 min; Temperature: 75 °C	Methane yield: 80% (v/v) after 40 days with ultrasound bath (PBU). Hydrogen yield decreased with ultrasonic probe (PSU).	[60]
Sugarcane Bagasse	Frequency: 35 kHz; Time: 1, 5, 10, and 15 min	Glucan conversion: 50% (26.35 g glucose/100 g biomass). Crystallinity index increased from 53% (raw) to 65% (3% NaOH + ultrasound). Lignin removal: ~50%. Ethanol yield: 5.6 g/L.	[61]

Table 2. Cont.

Biomass	Pretreatment Parameters	Main Results	Reference
Sugarcane Bagasse	Frequency: 40 kHz; Time: 35 min; Temperature: 30 °C	Maximum weight percent gain (WPG): 30.6% with 10 min ultrasound and maleic anhydride (1:1.1 <i>w/w</i>). FT-IR/NMR confirmed maleation at cellulose and hemicellulose. No impact on thermal stability.	[62]
Sugarcane Bagasse	Frequency: 24 kHz; Time: 45 and 60 min; Temperature: 50 °C	Maximum glucose yield: 69.06% theoretical; Pentose yield: 81.35%. Maximum ethanol yield: 91.8% theoretical (8.11 g/L). Low inhibitor formation (acetic acid: 0.95 g/L; furfural: 0.1 g/L).	[63]
Sugarcane Bagasse	Frequency: 24 kHz; Temperature: 22 °C	Glucose yield: 91.28% theoretical (38.4 g/L). Ethanol yield: 91.22% theoretical (17.9 g/L in 36 h). Lignin removal: 90.6%. Low inhibitor formation.	[64]
Sugarcane Bagasse	Frequency: 24 kHz; Time: 15, 30, and 45 min; Temperature: 40, 60, and 80 °C	Cellulose recovery: 95.78%. Delignification: 58.14%. Glucose production: 16.58 g/L. Xylose: 8.21 g/L. Crystallinity index decreased from 68.6% to 48.6%.	[65]
Sugarcane Bagasse	Frequency: 45 kHz; Temperature: 90 °C	Cellulose-to-reducing sugar conversion: 95.9% (12 h). Ultrasound-NMMO treated bagasse: 90.4% (24 h). Crystallinity index decreased (TCI: 1.393 → 0.878). Glucose production: 5.2 g/L.	[66]
Sugarcane Bagasse	Frequency: 20 kHz; Time: 180 s	Maximum sugar yield: 26.01 g/L (94.49% theoretical). Most influential factors: enzyme use, particle size, acid concentration, ultrasound duration, and power. Optimal acid: 3% (higher led to inhibitors). Optimal ultrasound: 120 W, 180 s.	[67]
Sugarcane Bagasse	Frequency: 24 kHz; Temperature: 70 °C	Lignin removal: 82.32%. Maximum reducing sugar yield: 96.27% theoretical. Cellulose recovery: 98.32%. Low inhibitor formation (acetic acid: 0.36 g/L).	[68]
Sugarcane Bagasse	Frequency: 20 kHz; Time: 40 min; Temperature: 55 °C	Lignin and hemicellulose increased by 2.2% and 1.3% (compared to non-ultrasound). Extracted lignin had high purity and better solubilization.	[69]
Corn Cob	Frequency: 20 kHz	Methane yield increased: 59.8% (VTS), 14.6% (corn cob) after isolated enzymatic hydrolysis. US + Hydrolysis improved methane yield by 41.8% (VTS) and 17.9% (corn cob).	[70]
Corn Cob and Corn Stalk	Frequency: 20 kHz; Time: 2 and 8 h; Temperature: 80 °C	Reducing sugar yield: Corn cob (scCO ₂ + US): 87.0% (+75% vs. control); Corn stalks (scCO ₂ + US): 30.0% (+13.4% vs. control). Ultrasound disrupted lignin and increased surface area. Ultrasound had a higher effect on corn cob than stalks.	[71]

Table 2. Cont.

Biomass	Pretreatment Parameters	Main Results	Reference
Soybean Straw	Time: 120–250 min; Temperature: 40–80 °C	Maximum reducing sugar yield: 53.27 mg (0.2 g soybean straw), 50.03 mg (0.2 g corn straw). Ultrasound improved biomass interaction with ionic liquids, enhancing cellulose dissolution.	[72]
Bermuda Grass	Frequency: 40 kHz; Time: 20–100 min; Temperature: 40–80 °C	Maximum reducing sugar yield: 36.89% (optimized). Optimal: 2% acid, 80 °C, solid–liquid ratio 12:1, ultrasound power 80 W, 100 min. Ultrasound reduced biomass crystallinity, increasing cellulose exposure.	[73]

In the context of the investigated biomasses, ultrasound is always conducted in an aqueous medium. Liu et al. [54] studied its effect on *Eucalyptus urophylla* × *E. grandis* and observed, through scanning electron microscopy (SEM), significant structural changes, including collapses and microchannels, that improved permeability. X-ray diffraction (XRD) revealed a 4% increase in crystallinity, attributed to the mechanical effects of ultrasound disrupting the lignin–carbohydrate matrix and exposing crystalline cellulose. Similarly, He et al. [56] reported structural damage and hydrogen bond disruption in cellulose, confirmed by Fourier-transform infrared spectroscopy (FTIR), indicating lignin removal. Comparing these results with Revin et al. [57], who applied ultrasound to *Pinus sylvestris* (27% lignin), differences emerged in lignin removal efficiency. Liu et al. [54] observed increased crystallinity and permeability in *Eucalyptus urophylla* × *E. grandis*, while Revin et al. [57] achieved a 61% increase in enzymatic hydrolysis yield in *Pinus sylvestris*, but required alkaline agents (NaOH, pyridine, ammonia) to enhance lignin removal. This suggests that high-lignin biomass requires chemical or combined pretreatment methods for effective lignin disruption.

Various chemical agents enhance ultrasound pretreatment. NaOH is the most commonly used, but acids such as HCl and acetic acid (CH₃COOH) are also effective. Additionally, advanced techniques like supercritical CO₂, eutectic solvents (e.g., ChCl/glycerol), and ionic liquids ([HMIM]Cl) have been combined with ultrasound to enhance delignification and cellulose exposure. Lignin content also influences operational parameters such as power, temperature, and duration. Liu and Wang [73] used a frequency of 40 kHz with diluted acid for Bermuda grass, significantly improving hydrolysis efficiency. Candido et al. [59] applied 20 kHz ultrasound with NaOH for sugarcane bagasse, achieving effective lignin removal. Higher power levels are generally required for high-lignin biomass like *Pinus sylvestris* (~27% lignin), aligning with Bussemaker and Zhang [48], who noted that lower ultrasound frequencies (~20 kHz) generate more intense cavitation, ideal for breaking biomass recalcitrance. Conversely, higher frequencies (40–45 kHz) produce more bubbles, enhancing chemical interactions. Liu et al. [62] demonstrated this effect in sugarcane bagasse pretreatment at 45 kHz, as did Liu and Wang [73] for Bermuda grass at 40 kHz.

The operation time for ultrasound pretreatment varies widely, from 60 s to 6 h, but most studies report durations between 15 and 60 min. This variation highlights the complexity of optimizing ultrasound pretreatment for different biomasses. Low frequencies (~20 kHz) are effective for high-lignin biomass, while higher frequencies (40–45 kHz), combined with diluted acids or other chemical agents, improve cellulose accessibility. Biomass with high lignin content, such as *Pinus sylvestris*, requires ultrasound and NaOH to achieve results comparable to those obtained with pure ultrasound in less lignified biomass like

Eucalyptus urophylla × *E. grandis* or Bermuda grass. Adjusting ultrasound parameters impacts saccharification efficiency and fermentable sugar release, reinforcing the need for tailored approaches based on biomass characteristics.

3.2.2. Microwave

The microwave pretreatment process uses non-ionizing electromagnetic radiation with frequencies between 300 MHz and 300 GHz, positioned between radio waves and infrared radiation [74,75]. Typically, 2.45 GHz is used in industrial applications due to its efficiency in dielectric heating [74]. This technique is based on dielectric heating induced by microwave application [76]. Unlike conventional conduction/convection heating, which relies on surface heat transfer [77], microwaves interact directly with biomass, resulting in rapid and volumetric heating [78,79].

The interaction of microwaves with lignocellulosic biomass involves differential heat absorption by polar components. Microwaves cause polar molecules like water to oscillate as they attempt to align with the alternating electromagnetic field. This movement generates friction between molecules, producing heat [78]. Microwaves penetrate biomass, heating its polar components and creating localized hotspots in the heterogeneous material [80]. This can trigger an “explosion” effect, facilitating the breakdown of recalcitrant structures. The electromagnetic field can also produce non-thermal effects, accelerating cellulose crystallinity reduction [81,82]. Table 3 shows these studies and the main parameters and results obtained.

Table 3. Studies on microwave pretreatment for biomass.

Biomass	Specific Parameters	Main Results	Reference
<i>Eucalyptus sawdust</i>	400 W, 15 min, 180 °C, 2.45 GHz	Sugar release: 3.5× higher vs. LHW; 100% sugar yield after enzymatic hydrolysis.	[83]
Corn stalks, Miscanthus	710 W, 300 W, 800 s (710 W), 180 s (300 W), Pressure (4 bars), 2.45 GHz	No significant increase in methane potential.	[84]
Sugarcane bagasse	600 W, 4 min	Reducing sugar yield: 0.665 g/g (NaOH), 0.83 g/g (NaOH + H ₂ SO ₄).	[85]
Napier grass	76 min, 147 °C	Maximum fermentable sugar yield: 14.38 g/L.	[86]
Sugarcane straw	1000 W, 2 min, 162 °C	Total sugar yield: 72.2%; Low inhibitor concentration.	[87]
Sugarcane trash, Oil Palm EFB	1200 W, 5 min, 180 °C	Reducing sugar yield: 0.33 g/g (sugarcane trash), 0.19 g/g (OPEFB).	[88]
Switchgrass, Miscanthus	800 W, 10 min, 60–210 °C	Increased subcritical water solubility by 7–10%.	[89]
Switchgrass, Big bluestem	450 W, 2.5 min	Increased total sugar recovery: 59.2% (switchgrass), 68.1% (big bluestem).	[90]
Pine chips, Beech chips, Hemp stems	300 W, 10–20 min, 140–180 °C	Sulfuric acid increased glucose release; nitric acid promoted xylose and galactose release.	[91]
Sugarcane bagasse	820 W, 3 min, NaOH 1%	Cellulose-enriched fractions resulted in the best ethanol yield.	[92]
Sugarcane leaf waste	400 W, 5 min	Lignin removal: 73%. Hemicellulose removal: 62%. Maximum ethanol yield: 31.06 g/L	[93]

Table 3. Cont.

Biomass	Specific Parameters	Main Results	Reference
Corn straw	640 W, 5 min	49.25% sugar yield after microwave pretreatment	[94]
<i>Pinus radiata</i>	50–150 °C, 50 min	Glucan digestibility: 79% after autohydrolysis (150 °C) and microwave (120 °C).	[95]
<i>Eucalyptus globulus</i> , <i>Pinus radiata</i>	80–120 °C, 50 min	Glucan digestibility increased: 68 g/100 g (eucalyptus), 78 g/100 g (pine).	[96]
Sugarcane bagasse	320 W, 3–10 min, 170 ± 5 °C	Glucose production: 64% after 7 min with H ₂ SO ₄ .	[97]
Sugarcane bagasse	10–20 min, 180–200 °C	Sugar yield: 44.9 g/100 g bagasse; ethanol yield: 35.8% (holocellulose fraction).	[98]
Corn straw	Up to 1600 W, 10 min, 120–210 °C	Methane production increased by 73.08% vs. control.	[99]
Elephant grass	200–600 W, 10–30 min	Lignin reduced to 3.24%, cellulose increased to 38.38%.	[100]

Understanding the interaction of microwaves with biomass is crucial for defining operational parameters such as power, exposure time, and temperature. Microwave pretreatment generally operates at 300 to 800 W, with exposure times between 2 and 20 min, depending on the biomass type and desired outcomes [86,93]. Higher power levels are associated with greater lignin removal and increased cellulose accessibility, as observed by Bichot et al. [84], who used 710 W on *Miscanthus* and corn stalks. However, if not adequately controlled, excessive power can increase the risk of inhibitor formation, such as furfural and HMF [85].

Like exposure time, temperature plays a crucial role in pretreatment. Binod et al. [85] demonstrated that 4 min at 600 W on sugarcane bagasse significantly increased fermentable sugar yield. In contrast, prolonged exposure (e.g., 20 min) caused excessive hemicellulose degradation, leading to inhibitor formation and reduced sugar yields [95]. Optimizing exposure time is essential to increase efficiency without compromising material quality. Temperature is also key, with 150 °C to 180 °C often reported as optimal for pretreatment. Lower temperatures result in less effective delignification, while around 180 °C enhances lignin removal [87]. However, exceeding 180 °C, especially under pressurized conditions, may improve pretreatment efficiency and raise the risk of thermal degradation and inhibitor formation [95]. Operational conditions, such as water or mild chemical solutions, influence pretreatment efficiency, particularly for specific biomasses. Water or a combination of diluted acids or alkalis enhances lignocellulose breakdown [85]. In sugarcane bagasse, Binod et al. [85] reported that microwave pretreatment significantly increased reducing sugar yields after enzymatic hydrolysis. Similarly, Bichot et al. [84] observed improved lignin removal and cellulose exposure in corn stalks and *Miscanthus*, reinforcing microwave pretreatment's potential to enhance saccharification and fermentation.

Microwave pretreatment effectiveness varies with biomass composition. For high-lignin biomass, such as *Pinus radiata* and *Eucalyptus globulus*, combining microwaves with ionic liquids or mild alkaline solutions was necessary for optimal results [96]. This suggests that more recalcitrant biomass requires more aggressive conditions [98].

3.2.3. Gamma Rays

Gamma-ray pretreatment involves high-energy electromagnetic radiation, which has a shorter wavelength than ultraviolet light and is highly penetrating [19,101]. This process is based on radiolysis, where gamma rays break down lignocellulosic biomass into smaller components, facilitating target compound extraction [46,102]. During this process, gamma rays penetrate the biomass, causing ionization and excitation of atoms, forming highly reactive free radicals that degrade the lignin–hemicellulose–cellulose matrix [46,103]. The energy released during radiolysis generates localized temperatures of up to 300 °C and pressure changes, effectively disrupting the complex lignocellulosic structure [104,105].

Several studies report findings on gamma-ray treatment of different biomasses. Al Gharib et al. [105] found that applying 1–2 MGy to woody biomass, such as pine wood, apple wood, and poplar, was sufficient for complete cellulose conversion to glucose after enzymatic hydrolysis. This study highlights the importance of high doses for dense biomass, where a more aggressive treatment is needed to fully break down the structure. The deep penetration of gamma radiation facilitated uniform degradation, emphasizing its effectiveness in biomass conversion.

Kapoor et al. [106] studied sugarcane bagasse pretreatment, confirming the efficacy of gamma radiation but with different results due to biomass type and process parameters. Applying 50 to 1000 kGy, they observed that gamma radiation was more effective than electron beams, particularly above 500 kGy, in breaking down cellulose and hemicellulose. However, lignin remained largely intact, requiring additional methods for effective treatment. This indicates that gamma radiation alone is effective but may need complementary techniques for complete biomass conversion.

Wu et al. [107] combined gamma radiation with ultrasound, reporting that 500 kGy and ultrasound intensification resulted in higher glucose yield in sugarcane bagasse pretreatment. The synergy between these methods compensated for individual limitations, such as gamma radiation's difficulty in fully degrading lignin. This combination could optimize fermentable sugar production without significantly increasing radiation doses, suggesting that integrating ultrasound enhances biomass treatment efficiency.

Li et al. [108] applied 100 to 500 kGy to sugarcane bagasse, showing that moderate doses altered the lignocellulosic structure and increased enzymatic accessibility. However, they detected inhibitors such as formic acid, acetic acid, and furfural, which increased proportionally with radiation dose. While these inhibitors did not significantly impact fermentable sugar production, higher doses required careful control to mitigate their effects on fermentation. These results underscore the importance of balancing radiation dosage to maximize sugar yield while minimizing inhibitor formation.

3.2.4. Electron Beam

Electron beam pretreatment uses high-energy ionizing radiation with low penetration but high dose rates, ranging from 3 MeV to 12 MeV [109,110]. Electron beam accelerators absorb electron energy as the material passes under or in front of the beam [111], altering chemical and biological bonds and inducing physical and chemical changes [110].

The treatment process ionizes atoms and molecules within the biomass, generating reactive species such as free radicals, cations, and anions [112]. These species interact with biomass components, modifying their structure and properties [113,114]. Electron beam exposure generates heat, triggering polymerization or degradation reactions [114].

The energy and dose of the electron beam are crucial for pretreatment efficiency. Al Gharib et al. [115] applied 1 to 3 MGy, achieving complete cellulose conversion to glucose. In contrast, Duarte et al. [116] used 50 kGy combined with hydrothermal treatment, obtaining a 74.72% glucose yield after 48 h. This comparison indicates that while high doses promote

complete conversion, combining electron beams with thermal treatments can produce significant results with lower radiation exposure.

Radiation dose also affects biomass structure. Guo et al. [117] found that 90 to 270 kGy, combined with inorganic salts such as MnCl_2 , FeCl_3 , and NaHCO_3 , facilitated hemicellulose and lignin removal, enhancing saccharification. This suggests that when combined with chemical agents, intermediate doses can efficiently degrade the lignocellulosic matrix.

Studies show that biomass type influences dose requirements. Al Gharib et al. [115] reported that 1 to 2 MGy was necessary for dense woods like pine and poplar, whereas sugarcane bagasse, studied by Rattanawongwiboon et al. [118], required only 50 to 200 kGy to generate reactive radicals. This highlights how biomass density and composition dictate optimal treatment parameters.

Karthika et al. [119] treated hybrid grass with doses up to 250 kGy, significantly reducing cellulose crystallinity—a key factor in enzymatic hydrolysis efficiency. While 75 kGy improved hydrolysis, 250 kGy resulted in a 79% reducing sugar yield in 48 h.

Shen et al. [120] demonstrated that low doses (2, 6, and 12 kGy) were sufficient for modifying corn starch, increasing its enzymatic hydrolysis susceptibility. This suggests that biomass with lower structural complexity can be effectively treated with lower radiation doses, saving energy while maintaining efficiency.

The formation of inhibitors during electron beam pretreatment can impact subsequent processes like fermentation. Guo et al. [117] observed organic acid generation when treating corn cob with 90 to 270 kGy, especially when combined with inorganic salts and H_2O_2 . However, efficient hemicellulose and lignin removal compensated for this disadvantage, improving enzymatic conversion. Similarly, Duarte et al. [116] reported that combining 100 kGy with acid hydrolysis in sugarcane bagasse minimized inhibitor formation. Rattanawongwiboon et al. [118] found that the sulfonation of biochar from irradiated bagasse (50 to 200 kGy) reduced toxic by-products, resulting in efficient sugar production with minimal inhibitor impact. These findings highlight that inhibitor formation, while a concern, can be controlled by optimizing radiation dose and treatment combinations.

3.3. Extrusion Pretreatment

Extrusion is a physical pretreatment that applies mechanical force and thermal energy, forcing the material through a die at high pressure and temperature, modifying its structure and composition [121]. It can be performed using single or twin-screw extruders [122]. Single-screw extruders have one solid screw, while twin-screw extruders consist of two intermeshing screws mounted on shafts [123].

During extrusion, lignocellulosic biomass undergoes shear, mixing, and heating, mainly in the mixing zone of the screw. This zone consists of successive kneading elements, connected with a slight offset angle [124]. These mechanical actions increase cellulose exposure, porosity, and surface area [125]. The literature presents several studies investigating extrusion as a pretreatment for fermentable sugar production. These studies are detailed in Table 4, including the respective operating conditions.

Table 4. Studies on extrusion pretreatment for biomass.

Biomass	Pretreatment Parameters	Main Results	Reference
<i>Eucalyptus grandis</i>	Twin-screw extruder, 150 rpm, 100 °C	Increased xylose release: 24.7–27.6 g/100 g (10% NaOH), 36.9–39.7 g/100 g (20% NaOH).	[122]
Eucalyptus wood chips	Twin-screw extruder	Maximum cellulose hydrolysis yield: 79.6% after 168 h enzymatic hydrolysis.	[123]

Table 4. Cont.

Biomass	Pretreatment Parameters	Main Results	Reference
<i>Eucalyptus grandis</i>	Twin-screw extruder, 100, 200, 300 rpm; 75, 100, 125 °C; (Liquid-to-solid ratio): Between 0.6 and 1.5. Moisture: 37–60%	Enhanced enzymatic digestibility: glucan (37.6%), xylan (74.6%) release.	[124]
Eucalyptus wood chips	Steam explosion through continuous screw extrusion (SESE)	Reduced fiber size, increased surface area, lignin depolymerization/repolymerization.	[125]
Eucalyptus wood chips	Twin-screw extruder, 45–120 rpm, ambient temperature	HCW + extrusion significantly increased glucose production. Monosaccharide yield: 39.3% of original wood weight.	[126]
Pine wood chips	Single-screw extruder, 100, 150, 200 rpm; 100, 140, 180 °C; Moisture: 25, 35, 45%	Higher screw speed and barrel temperature improved sugar recovery; high moisture reduced recovery.	[127]
Sugarcane Bagasse	Twin-screw extruder, 100 rpm, 130 °C, Moisture: 10%	Glucose yield increased by 330%, crystallinity reduced by 78%, improving enzymatic hydrolysis efficiency.	[128]
Sugarcane Bagasse and Straw	Twin-screw extruder, 20–150 rpm, 30–150 °C, Moisture: 10–12%	Glucose yield: 68.2% (straw, 7 passes), 36.6% (bagasse, 3 passes).	[121]
Sugarcane Bagasse	Twin-screw extruder, 15 rpm, 140 °C, Biomass pre-dried	Glucose yield: 91% (25% bagasse, 2 passes), 76.4% (50% loading).	[129]
Corn cob	Twin-screw extruder	Extrusion + NaOH + enzymatic hydrolysis increased methane production by 22.3% vs. raw cob digestion.	[130]
Soybean Hulls	Twin-screw extruder, 280, 350, 420 rpm; Barrel adjusted from 40 °C (inlet) to 80 °C (outlet); Moisture: 40%, 45%, 50%	Improved enzymatic hydrolysis: glucose yield increased by 155% vs. untreated biomass.	[131]
Soybean Hulls	Twin-screw extruder, 280, 350, 420 rpm; Drum: 80, 110, 140 °C (80 °C most efficient); Moisture: 0.4	Cellulose-to-glucose conversion: 94.8% (higher than acid: 69.2%, similar to alkali: 93.3%).	[132]
Soybean Hulls	Twin-screw extruder, 3.7 Hz (222 rpm); 110 °C; Moisture: 30–35%	Reducing sugars yield: 9–12%. Washing increased yield to 25–36%.	[133]
Grass	Twin-screw extruder	Increased biomethane potential (BMP): 11% (fresh grass), 18% (silage).	[134]
Switchgrass	Single-screw extruder, Compression ratios: 2:1 and 3:1; 50, 100, 150 rpm; 50, 100, 150 °C; Moisture: 15%, 25%, 35%, 45%	Maximum glucose recovery: 45.2% (15% moisture, 50 rpm, 150 °C, 3:1 compression).	[135]
Big bluestem	Single-screw extruder, 20–200 rpm; 45–225 °C; Moisture: 10–50%	Maximum sugar recovery: glucose (71.3%), xylose (78.5%), total sugars (56.9%).	[136]

Table 4. Cont.

Biomass	Pretreatment Parameters	Main Results	Reference
Prairie Cord Grass	Single-screw extruder, 20–200 rpm; 45–225 °C; Moisture: 75–78%	Maximum glucose (86.8%), xylose (84.5%), total sugars (82%) under optimized conditions.	[137]
Switchgrass, Prairie Cord Grass, and Big Bluestem	Single-screw extruder, 20–200 rpm; 45–225 °C; Moisture: 10–50%	Sugar recovery: Switchgrass (47.4%, torque: 85–100 Nm), Prairie Cord Grass (56.9%, torque: 27–42 Nm), Big Bluestem (56.9%, torque: 53–84 Nm).	[138]

Extrusion process parameters vary widely, directly impacting pretreatment efficiency. Screw speed significantly influences fermentable sugar yield. Karunanithy et al. [136] observed that increasing screw speed from 100 to 150 rpm raised cellulose recovery by 11% and hemicellulose by 7.7%. Similarly, Yoo et al. [132] found that lower temperatures (80 °C) were more efficient for soybean hull pretreatment. Fasheur et al. [128] reported that dry extrusion of sugarcane bagasse at 100 rpm and 130 °C improved saccharification efficiency due to increased enzymatic accessibility.

Besides screw speed and temperature, moisture content plays a crucial role. Karunanithy et al. [136] found that increasing moisture from 25% to 45% reduced cellulose recovery by 18% and hemicellulose by 34%. Moro et al. [121] showed that maintaining 10–12% moisture in bagasse and sugarcane straw, combined with glycerol as an additive, improved enzymatic accessibility, achieving 68.2% glucose yield for straw. These findings align with Lee et al. [126], who emphasized moisture control's role in enhancing enzymatic saccharification in eucalyptus chips.

The formation of inhibitors during extrusion is another critical aspect. Chemicals such as NaOH or H₂SO₄ can lead to salt or inhibitor formation, as observed by Doménech et al. [122] and Duque et al. [124]. However, most studies highlight that post-extrusion washing effectively removes these inhibitors. Duque et al. [124] noted that neutralization with H₂SO₄, followed by washing, significantly increased glucan and xylan yields. Similarly, Lamsal et al. [133] found that washing soybean hulls after extrusion raised reducing sugar yields from 9–12% to 25–36%, reinforcing the importance of this step in improving enzymatic saccharification efficiency.

3.4. Pulsed Electric Field Pretreatment

The pulsed electric field (PEF) method, initially used in molecular biology, has gained attention in food processing and biotechnology [139,140]. This pretreatment applies high voltage between two electrodes, with electric field strengths ranging from 0.1 to 80 kV/cm for 100 to 10,000 µs [14,19]. Its primary effect on lignocellulosic biomass is membrane disruption, increasing permeability [141]. The high-intensity electric field generates an electric potential across the membrane, leading to its rupture [142], facilitating enzyme, acid, and base penetration for cellulose hydrolysis [143,144].

PEF advantages include operation under ambient conditions, low energy consumption, and simplicity [145,146]. However, large-scale applications face challenges, such as the need for large pretreatment chambers, high pulse repetition rates, and increased current demands [140,147], requiring adaptations for improved efficiency [148].

Although PEF shows potential for lignocellulosic biomass pretreatment, its development is still incipient [147,149]. Kumar et al. [145] developed a PEF system for switchgrass and southern pine wood chips, observing increased dye absorption at 8 kV/cm, indicating enhanced biomass porosity and potential saccharification efficiency improvements.

Szwarc and Szwarc [148] investigated PEF in biogas production from corn silage, using 50 μ s rectangular pulses at -40 kV. A 4% glucose increase and 14% higher biogas yield were observed at 20 kV/cm with 180 s of pretreatment, but longer exposure showed no additional benefits, suggesting an optimal treatment duration.

Despite promising results, large-scale PEF adoption faces cost and scalability challenges [147]. To integrate PEF into industrial bioenergy conversion, refining field intensity, treatment time, and equipment design is essential. Identifying optimal parameters, such as the maximum effective treatment duration, is crucial to maximizing efficiency, as demonstrated by Szwarc and Szwarc [148].

4. Enzymatic Hydrolysis of Lignocellulosic Biomass After Physical Pretreatment

After lignocellulosic biomass pretreatment, enzymatic hydrolysis is a crucial step for producing fermentable sugars. This process breaks down cellulose and hemicellulose into glucose (C6) and xylose (C5), which serve as substrates for fermentation and value-added products [133,149].

4.1. Mechanism and Enzymatic Components

The main enzymes involved in lignocellulosic biomass conversion are cellulases and hemicellulases. The synergistic action of endoglucanase, exoglucanase, and β -glucosidase is essential for complete cellulose degradation into glucose [14,131]. Endoglucanase cleaves β -1,4 glycosidic bonds in amorphous cellulose regions, while exoglucanase acts at polymer ends, releasing cellobiose and celotriose, which β -glucosidase converts into glucose [150,151]. Hemicellulose structure varies by biomass type, with xylanase (endo-1,4- β -xylanase) as the predominant enzyme, breaking xylan glycosidic bonds to release xylo-oligosaccharides [152,153].

4.2. Effect of Pretreatment of Enzymatic Hydrolysis

Enzymatic hydrolysis efficiency depends on structural modifications induced by pretreatment, which enhance enzyme accessibility. Key factors include pH (4.8–5.5), temperature (45–55 $^{\circ}$ C), enzyme loading, and reaction time, ensuring enzyme stability and activity [87,93]. Physical pretreatment methods aim to enhance enzyme accessibility by modifying biomass structure, facilitating hydrolysis. The following sections discuss mechanical comminution, microwave, electron beam, and other methods, focusing on their impact on enzymatic conversion efficiency, challenges, and limitations.

4.2.1. Mechanical Comminution Pretreatment

Enzymatic hydrolysis following mechanical comminution varies significantly in glucose and xylose yields, influenced by milling conditions, enzyme types, and operational parameters. Inoue et al. [44] hydrolyzed ground eucalyptus with *Acremonium cellulolyticus* cellulase (40 FPU/g) at 45 $^{\circ}$ C, pH 5.0, for 72 h, obtaining 89.7% glucose and 72.5% xylose. These high yields reflect increased specific surface area and enzyme accessibility due to reduced cellulose crystallinity after ball milling.

Similarly, Sant'Ana da Silva et al. [41] studied sugarcane bagasse and straw hydrolysis using 15 FPU/g cellulase and 0.2% xylanase (OptimashTM BG, Genencor[®] International, Palo Alto, CA, USA), achieving 78.7% glucose for bagasse and 77.6% for straw. Compared to untreated samples, these results reinforce the importance of enzyme loading and optimized reaction times for maximizing saccharification. Zeng et al. [42] investigated corn residues with particle sizes ranging from 53–75 μ m to 425–710 μ m. Smaller particles favored glucose conversion, yielding 25.9% after 72 h with Spezyme CP (Sigma-Aldrich, St Louis, MO, USA), β -glucosidase (Sigma-Aldrich, St Louis, MO, USA) and Novozym 188 (Novozymes,

Denmark) at pH 4.8 and 50 °C. This highlights how particle size reduction enhances enzyme accessibility, improving hydrolysis efficiency. Zhu et al. [43] analyzed lodgepole pine subjected to disk milling, obtaining 49.3% glucose and 68% xylose. Compared to previous studies, these results suggest that woody biomasses, due to high lignin content and rigid structure, require specific milling adjustments to improve enzymatic digestibility.

However, mechanical comminution presents challenges for woody and dense biomasses, as their rigid structure and lignin content hinder enzymatic action. Optimizing particle size and equipment type is essential to enhance saccharification efficiency. Thus, mechanical comminution for rigid biomasses depends on advances in process control to overcome structural recalcitrance.

4.2.2. Ultrasound Pretreatment

Ultrasound-assisted pretreatment enhances enzymatic hydrolysis by disrupting biomass structure, reducing lignin content, and increasing cellulose accessibility, improving sugar yields. Studies highlight the impact of operational parameters, enzyme cocktails, and biomass characteristics on hydrolysis efficiency.

The choice of enzymatic cocktails significantly affects hydrolysis. Candido et al. [59] achieved 86.74% glucose conversion in sugarcane straw pretreated with ultrasound and NaOH using Celluclast (Millipore, Burlington, MA, USA) 1.5 L and β -glucosidase (Sigma-Aldrich, St Louis, MO, USA). Similarly, Velmurugan et al. [63] obtained 92.11% theoretical yield on ultrasound-pretreated sugarcane bagasse, underscoring the synergy between alkaline conditions and ultrasound in delignification and cellulose accessibility.

Acidic agents also enhance ultrasound pretreatment. Esfahani and Azin [67] reported a 94.49% theoretical yield using Cellubrix® (Novozymes A/S, Krogshoejvej, Bagsvaerd, Denmark) on sugarcane bagasse pretreated with dilute sulfuric acid and ultrasound, demonstrating the effectiveness of acidic catalysts in breaking down recalcitrant biomass structures.

Alternative biomass types benefit from ultrasound pretreatment. Yin et al. [71] obtained 42% glucose yield in corn cobs using Cellic enzymes (Novozymes, Denmark), while Liu and Wang [73] achieved 36.89% reducing sugar yield in Bermuda grass with ultrasound and diluted HCl, demonstrating its versatility across diverse feedstocks. Combining ultrasound with deep eutectic solvents (DES) has shown promise. Sharma et al. [58] reported 312 mg/g sugar yield in sugarcane bagasse using an *Aspergillus assiutensis* enzymatic cocktail, highlighting how ultrasound with DES enhances delignification, reduces cellulose crystallinity, and minimizes inhibitors.

Enzymatic loading and activity are key factors in hydrolysis efficiency. Revin et al. [57] achieved 61% glucose yield (35.5 g/L) in *Pinus sylvestris* using *Penicillium verruculosum* enzymes (204 U CMCase/g activity), illustrating how high enzyme activity combined with ultrasound improves digestibility, even in woody biomass. Despite its potential, biomass variability affects ultrasound pretreatment outcomes. High-lignin biomass (e.g., *Pinus sylvestris*) often requires aggressive chemical conditions or combined pretreatments, while low-lignin biomass (e.g., sugarcane bagasse and Bermuda grass) responds well to milder conditions.

Ultrasound pretreatment improves sugar yields across biomass types, but its success depends on optimizing process parameters, enzyme cocktails, and chemical combinations. Future research should expand ultrasound applications and refine pretreatment conditions for greater industrial scalability.

4.2.3. Microwave Pretreatment

The effectiveness of enzymatic hydrolysis after microwave pretreatment depends on enzyme type, loading, pH, temperature, and reaction time, which influence sugar yields and process efficiency. Most studies report pH 4.8–5.0 and temperatures of 45–50 °C, conditions typical for cellulose and hemicellulose hydrolysis in industrial applications [83,85,87,97].

Variations in enzyme loading have been observed with Accelerase 1500[®] (Genencor[®] International, Palo Alto, CA, USA) [95], Cellic[®] CTec2 (Novozymes, Denmark) [87,93], and Zytex cellulase (Zytex India Private Limited, Mumbai, India) [85]. Accelerase 1500[®] achieved 78% glucan digestibility in eucalyptus and pine [95], while Cellic[®] CTec2 (Novozymes, Denmark) yielded 72.2% sugarcane straw hydrolysis with low inhibitor formation [87]. These results align with studies demonstrating the synergy of cellulase and β -glucosidase in glucose and xylose release.

Hydrolysis times ranged from 24 to 72 h, depending on biomass recalcitrance. Binod et al. [85] obtained 0.83 g/g reducing sugars in 24 h using Zytex on microwave- and alkali-pretreated sugarcane bagasse, while Miranda et al. [92] required 72 h to achieve high glucose (46.25 mg/g) and xylose (190.43 mg/g) yields. These findings suggest that microwave pretreatment enhances enzymatic accessibility, reducing hydrolysis time, especially for more recalcitrant biomasses.

Significant differences in woody biomass vs. grasses were reported. Amini et al. [83] achieved 100% sugar conversion in *Eucalyptus regnans* treated at 180 °C for 30 min, while Rigual et al. [95] obtained 68 g/100 g glucan for eucalyptus and 78 g/100 g for pine, reinforcing the importance of optimized microwave conditions. Zhu et al. [97] confirmed that increased surface area and reduced cellulose crystallinity improve enzymatic accessibility, an effect also observed in switchgrass and big bluestem, where Karunanithy et al. [90] reported up to 83.2% yield.

Microwave combined with acidic catalysts (H_2SO_4 , $\text{Al}_2(\text{SO}_4)_3$) further increased glucose and xylose release, especially in woody biomass [88,91]. For sugarcane bagasse, Fonseca et al. [87] achieved 72.2% yield with Cellic[®] CTec2 (Novozymes, Denmark), emphasizing how microwave pretreatment facilitates fermentation. Additionally, Wang [99] reported that microwave-pretreated corn straw increased methane production by 73.08%, suggesting benefits for anaerobic digestion. However, applying microwaves to dense or woody biomass presents challenges due to high energy requirements and structural recalcitrance. Optimizing temperature and moisture is essential to avoid inhibitor formation (e.g., HMF), which can limit large-scale applications.

4.2.4. Electron Beam Pretreatment

The structure of biomass and process conditions significantly influence the effectiveness of enzymatic hydrolysis after electron beam pretreatment. Al Gharib et al. [105] reported 100% cellulose-to-glucose conversion using 1–2 MGy, where structural disaggregation enhanced enzyme accessibility. This efficiency gain is attributed to reduced cellulose crystallinity and increased surface area. Hydrolysis was performed with a mixture of endo 1,4 β -glucanase (2 U mg^{-1}), Exo 1,4 β -glucanase (cellobiohydrolase), and β -glucosidase, with 40–100 μL of enzyme solution per 400–600 mg of irradiated biomass.

Studies on gamma radiation pretreatment, such as Wu et al. [107], support these findings. Their glucan-to-glucose conversion exceeded 70% with 15 FPU/g enzyme loading at 50 °C, pH 4.8—conditions ideal for commercial cellulases [102]. However, electron beam treatment modifies biomass structure more efficiently, potentially reducing enzyme requirements.

Enzyme loading must be optimized according to pretreatment intensity and biomass structure. Biomass treated with 800 kGy showed higher enzymatic accessibility due to

reduced crystallinity, requiring lower enzyme doses for high glucose yields [106]. However, high radiation doses can generate inhibitors like furfural and acetic acid, which compete with enzymes and reduce process efficiency, emphasizing the need for careful pretreatment control [108]. Additionally, high doses increase operational costs, limiting industrial feasibility unless strategies for inhibitor dilution or controlled radiation dosage are implemented.

4.2.5. Gamma Rays Pretreatment

Enzymatic hydrolysis of gamma radiation-pretreated biomass is a promising method to enhance cellulose and hemicellulose conversion into fermentable sugars, primarily glucose. Wu et al. [107] demonstrated that glucan-to-glucose conversion exceeded 70% with 15 FPU/g enzyme loading at 50 °C and pH 4.8, highlighting the role of pretreatment in modifying biomass structure and increasing enzyme accessibility.

This process relies on cellulolytic enzymes (cellulase, hemicellulase, and β -glucosidase), which degrade cellulose and hemicellulose into monosaccharides. The effectiveness of enzymatic hydrolysis depends on structural modifications achieved during pretreatment. Al Gharib et al. [105] reported 100% cellulose-to-glucose conversion in electron beam-treated biomass (1–2 MGy), where structural disaggregation significantly enhanced enzymatic accessibility. While electron beam treatment induces intense modifications, gamma radiation reduces cellulose crystallinity more gradually, allowing dose adjustments based on biomass type.

Optimizing enzyme loading, pH, and temperature enhances glucose conversion. Duarte et al. [113] combined electron beam and hydrothermal pretreatment, achieving 74.72% glucose yield in 48 h, demonstrating the synergy between methods. In gamma radiation pretreatment, precise parameter control and complementary enzymes improve efficiency. Lower gamma radiation doses can be sufficient for less complex biomasses. Shen et al. [117] showed that 2–12 kGy effectively altered corn starch structure, increasing hydrolysis rates with lower enzyme loading, indicating that dose adjustments tailored to biomass type can enhance efficiency and reduce costs.

However, costs and inhibitor formation remain challenges similar to electron beam pretreatment. Mitigation strategies and the use of enzyme cocktails tolerant to inhibitors are crucial for industrial feasibility.

4.2.6. Extrusion Pretreatment

Extrusion enhances biomass accessibility to enzymatic hydrolysis by increasing surface area and reducing cellulose crystallinity. Yoo et al. [132] reported 95% glucose yield from soybean hulls extruded at 40% moisture and 350 rpm screw speed, compared to 69.6% in untreated hulls, highlighting the importance of optimizing mechanical conditions for cellulose conversion, especially in high-density biomass.

The enzyme cocktail selection significantly impacts hydrolysis efficiency. Yoo et al. [131] achieved 87% cellulose-to-glucose conversion in extruded soybean hulls using cellulase and β -glucosidase, increasing to 155% with additional cell wall-degrading enzymes. Similarly, Lamsal et al. [133] reported 12–36% sugar yields from extruded wheat bran, demonstrating the importance of hemicellulases in hemicellulose-rich substrates.

Moisture and temperature play a crucial role in enzymatic hydrolysis. Karunanithy et al. [135] found that increasing moisture from 25% to 45% reduced cellulose recovery by 18% and hemicellulose by 34%, as excess moisture dampens heat and shear effects. In contrast, Moro et al. [121] showed that 10–12% moisture, combined with glycerol, increased glucose yield by 68.2% in sugarcane straw, reinforcing the need for precise moisture control based on biomass type. Biomass density also affects enzyme choice and extrusion

conditions. Tian et al. [123] obtained 79.6% glucose yield in eucalyptus wood after hot water extraction and mechanical extrusion, whereas lower-density materials, such as wheat bran, required less shear energy to achieve 25–36% reducing sugar yield.

Inhibitor control is essential for optimal hydrolysis. Doménech et al. [122] demonstrated that post-wash steps reduce inhibitors such as HMF and organic acids, while Duque et al. [124] found that washing extruded sugarcane bagasse significantly improved glucan and xylan yields. However, precise control of moisture and temperature remains a challenge, particularly in high-density biomass, requiring pressure and speed adjustments. These technical demands necessitate biomass-specific optimizations, which may limit large-scale applications.

4.2.7. Pulsed Electric Field Pretreatment

Pulsed electric field (PEF) pretreatment enhances biomass porosity and cellular permeability, theoretically facilitating enzymatic hydrolysis. This method applies high-intensity electric pulses to reorganize cellular structures, increasing fiber exposure for enzymatic action. Kumar et al. [145] reported that PEF modifies biomass structure, making it more susceptible to degradation and improving fermentable sugar conversion rates.

However, the direct impact of PEF on enzymatic hydrolysis remains underexplored. While studies document structural changes, such as increased porosity and permeability, few directly assess monosaccharide release after hydrolysis. This limitation suggests that further studies are needed to validate the practical feasibility and efficiency of PEF in enzymatic hydrolysis.

5. Biomass Final Treatments

The pretreatment process plays a crucial role in breaking down the complex structure of biomass, making fermentable sugars more accessible for conversion into bioethanol, biogas, and other valuable compounds. Each method offers distinct advantages and challenges, influencing the efficiency of product yields and the overall feasibility of large-scale industrial applications.

5.1. Bioethanol

Bioethanol production is one of the main applications of fermentable sugars released after biomass pretreatment. In mechanical comminution, Inoue et al. [44] reported that ball-milled eucalyptus wood achieved over 90% glucose-to-ethanol conversion in 24 h, highlighting its efficiency in releasing fermentable sugars. Similarly, Sant'Ana da Silva et al. [41] achieved nearly 90% conversion yields from sugarcane bagasse hydrolysates fermented with *Saccharomyces cerevisiae*, demonstrating the advantage of high-purity hydrolysates free from fermentation inhibitors.

Ultrasound pretreatment has also improved ethanol production. Revin et al. [57] reported that *Pinus sylvestris* pretreated with ultrasound yielded 3.11% (*v/v*) ethanol, a significant increase attributed to enhanced glucose release. Candido et al. [60] demonstrated that sugarcane straw pretreated with ultrasound in an alkaline medium generated glucose-rich hydrolysates, confirming the industrial viability of this approach. Eblaghi et al. [61] further validated these findings, showing that ultrasound combined with alkaline pretreatment improves sugar release and fermentation efficiency.

Microwave pretreatment has proven particularly effective in accelerating hydrolysis and ethanol production. Miranda et al. [97] demonstrated that microwave-assisted alkali and acid treatments of sugarcane bagasse significantly increased ethanol yields, improving cellulose accessibility. Similarly, Binod et al. [85] observed that NaOH and microwave pretreatment facilitated fermentable sugar release, enhancing ethanol production.

Gamma radiation pretreatment has shown promising results. Al Gharib et al. [105] reported that pine and poplar treated with 1 MGy achieved complete cellulose-to-glucose conversion, enabling efficient ethanol fermentation with *S. cerevisiae*. Kapoor et al. [106] achieved high ethanol yields from sugarcane bagasse pretreated with gamma radiation, optimizing production through simultaneous saccharification and fermentation (SSF). However, gamma radiation can generate inhibitors, such as furfural and acetic acid, which negatively impact fermentation efficiency [108].

Electron beam pretreatment is still under evaluation for large-scale bioethanol production. Al Gharib et al. [105] reported high-purity glucose hydrolysates from electron beam-treated woody biomass, but their fermentation efficiency remains untested at an industrial level. Rattanawongwiboon et al. [115] showed that electron beam-treated sugarcane bagasse could be converted into sulfonated biochar, efficiently producing fermentable sugars, but further research is needed to confirm its viability for ethanol production.

Extrusion pretreatment has demonstrated efficiency in bioethanol production. Duque et al. [124] reported successful glucose and xylose conversion after alkaline extrusion pretreatment, while Tian et al. [123] achieved 55.3% ethanol yield (15.4 g/L) in 48 h from extruded eucalyptus wood chips, demonstrating its feasibility for recalcitrant woody biomass.

Producing bioethanol from lignocellulosic biomass requires careful evaluation of pretreatment methods, considering biomass characteristics, process efficiency, and operational costs. While all techniques offer significant advantages, selecting the optimal approach depends on the desired product yield and industrial feasibility.

5.2. Biogas

Biogas production is an essential application of lignocellulosic biomass, particularly for renewable energy generation. After enzymatic hydrolysis, fermentable sugars undergo anaerobic digestion to produce biogas, mainly methane. Various pretreatment methods influence biogas yields, depending on biomass type, pretreatment conditions, and operational parameters.

Mechanical comminution increases biomass surface area, improving microbial accessibility in anaerobic digestion. Sant'Ana da Silva et al. [41] observed that hydrolysates from sugarcane bagasse pretreated by comminution were free from inhibitors, facilitating efficient fermentation. Although this study focused on ethanol production, the increased surface area likely enhanced biogas yields. However, high energy consumption remains a major challenge, limiting large-scale applications.

Ultrasound pretreatment improves digestibility by breaking cell walls and enhancing sugar accessibility. Pérez-Rodríguez et al. [70] found that ultrasound combined with enzymatic hydrolysis increased methane yields by 22.6% in corn cob and vine trimming shoot hydrolysates. Similarly, Martínez-Jiménez et al. [60] achieved 68% methane yields from sugarcane straw hydrolysates, demonstrating ultrasound's effectiveness in bioenergy applications.

Microwave pretreatment enhances biomass digestibility and biogas production. Ude [101] showed that microwave-treated elephant grass produced 15% more methane than untreated samples. However, Bichot [84] found that microwaves were less effective than conventional methods for corn stalks and miscanthus, suggesting that biomass composition strongly influences pretreatment success.

Gamma radiation pretreatment improves biogas production, particularly for agricultural residues. Wu et al. [107] reported that combining gamma radiation with ultrasound increased methane yields by 30%, achieving 72% energy recovery. This synergy enhances digestion efficiency, especially for recalcitrant biomass. However, inhibitor formation (e.g., furfural, acetic acid) remains a concern, requiring post-treatment strategies.

Electron beam pretreatment primarily improves cellulose accessibility for enzymatic hydrolysis. Rattanawongwiboon et al. [115] studied electron beam-treated sugarcane bagasse converted into sulfonated biochar, enhancing fermentable sugar production. Although this study focused on sugar yields, the increased digestibility suggests a potential boost in biogas production. However, more research is needed on its direct impact on anaerobic digestion.

Extrusion pretreatment has shown promise for bioethanol and biogas production. Pérez-Rodríguez et al. [130] found that extrusion followed by alkaline hydrolysis increased methane content to 65.6%, a 22.3% improvement over untreated corn cob. Souza et al. [134] demonstrated 18% higher biogas yields from fresh and ensiled grass, confirming extrusion's potential to enhance anaerobic digestion efficiency.

The effectiveness of each pretreatment method depends on biomass type, operational conditions, and inhibitor formation. While ultrasound, microwave, gamma radiation, and extrusion show potential for increasing methane yields, scalability challenges remain. Further research should focus on optimizing these methods and integrating them into biorefineries to maximize economic and environmental benefits.

5.3. Other Products Obtained

In addition to bioethanol and biogas, lignocellulosic biomass offers potential for producing high-value products across industries such as energy, food, plastics, and chemicals. Several studies highlight the diverse applications of biomass-derived products.

Butanol, a second-generation biofuel, is a promising alternative due to its higher energy content and superior engine compatibility compared to ethanol. Buaban et al. [40] investigated butanol production from pentoses and non-hexose sugars derived from ball-milled sugarcane bagasse, though specific yields were not reported. This underscores the potential of pentose-based biofuels, an area requiring further research.

Butyric acid is another valuable product, widely used in the food and bioplastics industries. Fermentable sugars from biomass hydrolysates enable its production. Buaban et al. [40] mentioned butyric acid formation from fermenting pentoses, highlighting its industrial relevance. Additionally, butyric acid can be converted into bioGLP (biogasoline-like products), expanding its applications.

Acetic acid can also be obtained from biomass pretreated with gamma radiation. Wu et al. [107] reported that gamma radiation combined with ultrasound increased acetic acid yields, achieving 20.1 g/L from rice straw and corn stalks. This simultaneous increase in acetic acid and methane yields suggests that combined pretreatments enhance biofuel and chemical production.

Bioplastic production from lignocellulosic biomass has gained attention. Rattanawongwiboon et al. [115] explored the conversion of electron beam-treated sugarcane bagasse into sulfonated biochar, which acted as a catalyst for producing fermentable sugars suitable for bioplastics. Though the study focused on sugar recovery (94.5%), further research is required to quantify actual bioplastic yields.

Hydrogen is a clean energy source with great potential when derived from biomass. Martínez-Jiménez et al. [60] examined hydrogen and biomethane production from sugarcane straw hydrolysates, achieving 7.1 g/L of hydrogen and 68% methane yield. Additionally, Irmak [89] used aqueous phase reforming (APR) to produce 3.5 g/L of hydrogen from switchgrass and miscanthus hydrolysates, reinforcing biomass's versatility for hydrogen generation.

Beyond these biofuels, sucrose, ethylene glycol, and succinic acid have also been investigated. Zhu et al. [43] suggested that lodgepole pine hydrolysates pretreated via disk milling could synthesize ethylene glycol and succinic acid, though further optimization is

needed to quantify yields. These compounds have broad applications in plastics and the chemical industry.

In summary, lignocellulosic biomass offers many alternative products, including butanol, butyric acid, bioplastics, hydrogen, acetic acid, and chemical compounds like ethylene glycol and succinic acid. These products expand the range of biomass applications and open the door to integrated biorefineries that can generate various sustainable, high-value products.

6. Energy and Costs Associated with Physical Pretreatment

Energy and cost analysis are essential for evaluating the feasibility of physical pretreatment methods in converting lignocellulosic biomass into fermentable sugars. Optimizing these factors in an industrial context determines economic efficiency and impacts biorefinery's environmental sustainability.

6.1. Mechanical Comminution

Physical pretreatment methods, such as ball milling and disk milling, play a key role in reducing the recalcitrance of lignocellulosic biomass. However, their industrial application is constrained by high energy consumption and associated costs.

Inoue et al. [44] investigated the energy requirements of ball milling under laboratory and industrial conditions. Ball milling consumed 108 MJ/kg of wood in lab-scale experiments over 120 min. At an industrial scale, this was reduced to 31 MJ/kg for a plant processing 100 dry tons/day, applying a scale efficiency factor of 0.2. Despite this improvement, ball milling remains energy-intensive, limiting its standalone feasibility for large-scale operations. The authors proposed combining ball milling with hot-compressed water (HCW) pretreatment, which reduced energy consumption to 6.97 MJ/kg when applying HCW at 160 °C for 30 min, followed by 20 min of ball milling, demonstrating the value of integrated methods.

Zhu et al. [43] assessed disk milling energy consumption, reporting 699 kWh/ton for untreated wood. However, using SPORL pretreatment (sulfite pretreatment to overcome recalcitrance of lignocellulose) reduced this to 153 kWh/ton, a 78.1% reduction, improving substrate digestibility while lowering costs. Sant'Ana da Silva et al. [41] evaluated wet disk milling, reporting 48 MJ/kg for sugarcane bagasse and 39.6 MJ/kg for sugarcane straw. Although less energy-intensive than ball milling, damp disk milling resulted in lower glucose conversion rates. However, it avoided fermentation inhibitors, offering an environmentally friendly alternative. Further optimization is recommended to enhance enzymatic yields while reducing energy demands.

Buaban et al. [40] acknowledged the high energy intensity of ball milling but emphasized its effectiveness in converting crystalline cellulose into amorphous forms, enhancing enzymatic hydrolysis. However, diminishing returns with extended milling times, such as four hours, indicate the need to balance energy input and yield improvements.

Mechanical comminution methods improve biomass digestibility but remain energy-intensive, limiting industrial viability. Studies highlight the importance of integrated processes and energy-efficient equipment to enhance economic feasibility and sustainability.

6.2. Ultrasound

The reviewed studies provided limited data on the energy consumption and costs of ultrasound pretreatment, except for Velmurugan and Muthukumar [68], who focused on sugarcane bagasse. They reported that the energy requirements for ultrasound pretreatment were 72 MJ/kg, significantly lower than steam explosion (99 MJ/kg) and autoclave methods (233 MJ/kg), positioning ultrasound as a more energy-efficient alternative. Similarly,

Hu et al. [72] described ultrasound as a low-energy method compared to conventional soybean and corn straw approaches. However, they did not include quantitative energy data or detailed cost analyses.

Ultrasound efficiency depends on operational parameters, such as power, frequency, exposure time, and the solid-to-liquid ratio (SLR). Higher power levels improve biomass structural disruption and lignin removal but proportionally increase energy consumption. For example, Liu et al. [54] applied ultrasound to eucalyptus wood for up to six hours, demonstrating wide variation in treatment durations that affect process efficiency and energy use. Given the variability in operational conditions, energy assessments must be tailored to specific biomass types and configurations. Systematic evaluations are needed to identify optimal, cost-effective configurations to advance ultrasound pretreatment for industrial applications.

6.3. Microwave

Microwave pretreatment offers potential advantages, but these depend on process parameters, biomass type, and application scale. Zhu et al. [97] reported a 5.7-fold reduction in processing time using sulfuric acid-assisted microwave pretreatment, significantly lowering energy consumption compared to conventional heating. Ude et al. [100] found that microwave pretreatment of elephant grass reduced hydraulic retention time in anaerobic digestion, indirectly minimizing energy demands. Similarly, Kłosowski et al. [91] highlighted that microwave usage is more energy-efficient than conventional methods. However, high power levels can undermine economic benefits if not optimized, as observed by Binod et al. [85] and Karunanithy et al. [90].

Catalyst usage also influences costs. For instance, Fonseca et al. [87] and Miranda et al. [92] enhanced sugar yields with acidic solutions, increasing material costs. Alternatively, Sasaki et al. [98] applied biological pretreatment before microwave application, reducing chemical usage but extending processing times. Despite the potential for energy savings, scaling microwave-assisted pretreatment remains challenging. Optimizing process parameters and integrating synergistic methods are critical to ensuring economic viability at industrial scales.

6.4. Gamma Radiation

Gamma radiation pretreatment enhances biomass digestibility but faces challenges related to energy consumption and operational costs. These costs stem from maintaining cobalt-60 sources, irradiator efficiency, and cooling systems [105]. While gamma radiation eliminates chemical reagents and extensive post-treatment, it involves high energy demands. Wu et al. [107] noted significant energy savings in grinding irradiated residues, offsetting initial irradiation costs. Biomass densities and recalcitrance levels influence energy needs, with woody biomass requiring higher doses (1–2 MGy) than agricultural residues like sugarcane bagasse (100–800 kGy). Although gamma radiation avoids energy-intensive heat treatments, its high operational costs necessitate innovations in irradiator design and process integration to enhance feasibility.

6.5. Electron Beam

Electron beam pretreatment modifies lignocellulosic biomass structures but requires substantial energy for electron accelerators operating at 3–12 MeV [105,117]. The doses needed (90 kGy to 3 MGy) depend on biomass type, with woody materials demanding higher energy due to more remarkable recalcitrance. Integrating electron beams with other methods, such as hydrothermal pretreatment, may reduce energy consumption, though detailed quantitative data are lacking. Advances in equipment efficiency and parameter optimization are essential for industrial scalability.

6.6. Extrusion

Extrusion pretreatment enhances enzymatic accessibility but presents challenges related to energy consumption. Operational parameters like screw speed, temperature, and moisture content significantly affect energy demands. Karunanithy et al. [136] reported that increasing screw speed and temperature improved sugar recovery but proportionally raised energy consumption. Moro et al. [121] observed specific energy consumption (SEC) ranging from 20 kJ/g to 123 kJ/g for sugarcane residues under different settings, highlighting the importance of parameter optimization. Moisture content also affects energy efficiency. Higher moisture levels reduce shear force and heat generation, increasing energy demands. Tailoring extrusion settings for specific biomass types and integrating renewable energy sources can mitigate costs, making the process more viable for industrial applications.

6.7. Pulsed Electric Field (PEF)

PEF pretreatment offers low energy requirements under controlled conditions, disrupting cell membranes with moderate electric field intensities [145]. However, industrial scaling challenges include larger chambers and higher current demands, which increase energy consumption [147]. Optimizing treatment parameters, such as pulse duration, is critical to avoiding unnecessary energy use. While PEF shows promise at the laboratory scale, innovations in equipment design and cost assessments are needed for industrial feasibility. These advancements will be crucial to realizing PEF's potential as an energy-efficient pretreatment method.

7. Conclusions and Critical on Physical Pretreatment

Developing efficient and sustainable strategies to address the recalcitrance of lignocellulosic biomass is crucial for advancing modern biorefineries. While the physical pretreatment techniques explored in this review have achieved significant progress in enhancing biomass accessibility and boosting sugar yields, several challenges that limit their industrial scalability remain. Among these are high energy requirements, the complexity of optimizing operational parameters, and the need to tailor processes to accommodate the natural variability in biomass composition.

A deeper examination of these methods reveals that no single solution fits all scenarios. Each technique has unique strengths and limitations, often determined by the type of biomass and the desired end products. This reinforces the need for continuous research to understand these methods better and to develop innovative combinations. Hybrid approaches that combine the benefits of multiple technologies are gaining recognition as promising solutions for efficient biomass deconstruction while addressing energy and cost concerns.

Economic and environmental sustainability are critical considerations for these methods. Advances in energy optimization and cost reduction are essential to improve feasibility. Integrating renewable energy sources and repurposing by-products within circular bioeconomy frameworks can further enhance sustainability. Moreover, scaling laboratory successes to industrial applications will be key to ensuring both effectiveness and commercial viability.

7.1. Common Aspects of Physical Pretreatment Technologies

7.1.1. Structural Modification

All methods modify the structure of lignocellulosic biomass to improve enzymatic digestibility—techniques like extrusion and ultrasound create microchannels, exposing cellulose fibers. In contrast, gamma radiation and electron beam methods disrupt dense

biomass structures. These changes increase surface area and porosity, improving fermentable sugar production.

7.1.2. Energy Demands

Energy consumption is a common challenge, particularly for methods involving high temperatures or specialized equipment. Optimizing parameters such as moisture content, processing speed, and temperature can help mitigate these costs. For instance, ultrasound has demonstrated lower energy requirements compared to methods like autoclaving or hydrothermal treatment.

7.1.3. Adaptability to Biomass Variability

The efficiency of each technology depends on the type of biomass being processed. Gamma radiation is highly adaptable to different substrates, while extrusion requires specific adjustments to optimize sugar recovery in dense or lignin-rich biomass. Customizing pretreatment strategies to match biomass characteristics is essential.

7.1.4. Generation of Inhibitors

Some methods can form inhibitory compounds, such as HMF and organic acids, especially when aggressive chemical agents are involved. However, this is not a universal issue. Ultrasound and electron beam pretreatments, when applied without chemical additives, typically result in fewer inhibitors, offering a significant advantage for subsequent biological processes. Additionally, post-treatment washing, commonly used in extrusion, helps minimize the impact of inhibitors.

7.2. Mechanical Comminution

Mechanical comminution is an essential step in processing lignocellulosic biomass, focusing on particle size reduction and increased surface area to enhance the effectiveness of subsequent pretreatment and conversion processes. While its isolated effects are often not deeply studied, earlier research emphasizes its potential for high saccharification yields, particularly in overcoming physical recalcitrance [41,44]. However, challenges related to extended processing times and high energy consumption persist, especially when working with biomass rich in lignin or crystalline cellulose. Despite these challenges, mechanical comminution remains a critical part of biomass processing. By ensuring particle uniformity, it facilitates compatibility with downstream operations, contributing to overall process efficiency. Most studies consider it a preparatory technique, processing biomass to particle sizes generally below 2 mm to enhance reactivity and enable the efficient application of other pretreatment methods.

7.3. Ultrasound

Ultrasound pretreatment has demonstrated significant potential in enhancing the enzymatic digestibility of lignocellulosic biomass. By leveraging cavitation and sonochemical effects, ultrasound effectively disrupts recalcitrant structures, increasing cellulose accessibility and sugar yields. This method induces notable microstructural changes, such as forming microchannels and removing lignin, across various biomass types, including sugarcane bagasse, straw, and woody materials like *Eucalyptus grandis* × *E. urophylla* [47–49].

A key advantage of ultrasound is its minimal inhibitor formation during standalone pretreatment, facilitating subsequent enzymatic hydrolysis. However, when combined with chemical agents under harsher conditions, there is a risk of inhibitor generation, requiring precise parameter optimization to minimize adverse effects [51,52]. Studies have shown synergistic benefits in these combined approaches, further emphasizing ultrasound's potential in integrated pretreatment strategies [53,54].

Despite these advantages, energy consumption and cost challenges remain critical for scaling ultrasound to industrial applications. Velmurugan and Muthukumar (2012) [64] highlight ultrasound as an energy-efficient alternative to steam explosion and autoclaving methods. However, the lack of standardized benchmarks hinders a comprehensive comparison of its industrial competitiveness. While numerous studies have explored biomass types like sugarcane bagasse and Eucalyptus, research on energy crops remains limited. Notably, soybean hulls—an important biomass in countries like Brazil—are underrepresented, indicating a significant gap in the literature.

In conclusion, ultrasound pretreatment is a promising and versatile method for enhancing enzymatic hydrolysis. However, optimizing operational parameters, validating energy and cost metrics, and exploring underrepresented biomass types, such as soybean hulls, are essential steps for establishing ultrasound as a sustainable and competitive technology for industrial applications.

7.4. Microwave

Microwave-assisted pretreatment demonstrates significant potential to enhance the enzymatic digestibility of lignocellulosic biomass. Its rapid heating and efficient structural disruption offer clear advantages, particularly for agricultural residues such as sugarcane bagasse and energy grasses like switchgrass [88,98]. In many cases, low levels of inhibitor formation facilitate downstream fermentation processes, making it an appealing method for integrated biorefineries. However, more recalcitrant biomass types, such as hardwoods, often require harsher conditions or catalysts, which can increase energy demands and costs [98].

Despite advancements, critical gaps persist in the literature. For example, while energy crops like sugarcane have been extensively studied, biomass such as soybean hulls remains largely unexplored. Given the economic relevance of soybeans in countries like Brazil, exploring these underutilized materials could unlock new industrial applications.

Energy consumption and cost optimization remain significant challenges. Microwave pretreatment has demonstrated energy efficiency and reduced processing times compared to conventional methods [97]. However, the economic feasibility of standalone microwave use depends on further optimization. Eliminating the need for catalysts could reduce costs but might limit efficiency for denser biomass. Alternatively, combining microwaves with methods such as extrusion or biological treatments shows promise in mitigating costs, though scalability and industrial viability require further validation [90,98].

Another critical aspect is scaling microwave pretreatment for industrial applications. While laboratory results are promising, few studies have evaluated its integration into biorefineries or its ability to handle large biomass volumes efficiently. Addressing this gap is essential to determine its practicality in industrial settings.

7.5. Gamma Rays

Gamma radiation pretreatment holds significant promise for enhancing the digestibility of lignocellulosic biomass by breaking down recalcitrant structures and increasing enzymatic accessibility. Its ability to penetrate dense materials uniformly and operate without chemical reagents distinguishes it as a sustainable method for biomass processing. Studies such as Al Gharib et al. [105] and Wu et al. [107] have demonstrated its effectiveness in converting various biomass types, from woody materials to agricultural residues, into fermentable sugars. However, energy consumption and operational cost challenges must be addressed for broader adoption.

The infrastructure required for gamma radiation, including cobalt-60 sources and shielding systems, contributes to high initial investments and maintenance expenses. Com-

parisons with methods like electron beam and hydrothermal pretreatment reveal that while gamma radiation offers superior penetration and uniform treatment, its energy demands are often higher. Kapoor et al. [106] emphasized that continuous operation for large-scale biomass processing highlights the need for innovations to enhance energy efficiency and cost-effectiveness.

Hybrid approaches provide a pathway to mitigate these limitations. Combining gamma radiation with techniques such as ultrasonic or alkaline treatments has shown potential to reduce energy demands while maintaining or enhancing biomass digestibility. Wu et al. [107] reported significant improvements in enzymatic hydrolysis yields through a combined gamma radiation and ultrasound approach, underscoring the benefits of integrating technologies to optimize pretreatment processes.

Future research must prioritize innovations that improve energy efficiency and reduce fixed costs, such as advancements in cobalt-60 recycling and developing more efficient irradiators. Incorporating gamma radiation as a step within multi-purpose biorefineries could also distribute energy costs across multiple products, enhancing its industrial viability. Moreover, valorizing by-products like lignin and biochar within circular bioeconomy frameworks could improve this technology's sustainability and profitability.

Despite its potential, certain biomass types remain underexplored. While studies on agricultural residues and woody materials are common, hardwood biomass and soybean residues—particularly relevant in regions like Brazil—are scarcely investigated. Addressing these gaps through targeted research could expand the applicability of gamma radiation and unlock new opportunities for sustainable biomass processing.

Gamma radiation pretreatment offers a promising pathway for sustainable biomass utilization. Future efforts should address scalability challenges, optimize dose requirements, and integrate gamma radiation into circular bioeconomy models. By overcoming these technical and economic barriers, gamma radiation could become a cornerstone technology in advancing bio-based industries.

7.6. Electron Beam

Electron beam pretreatment enhances the enzymatic digestibility of lignocellulosic biomass by inducing structural disorganization, reducing cellulose crystallinity, and minimizing inhibitor formation. Studies have demonstrated its effectiveness across various biomass types [106,116]. However, challenges such as high energy consumption and scalability constraints limit its widespread adoption in biorefineries [113,117]. High energy demands, particularly for dense biomass like hardwoods, highlight the necessity of process optimization. Strategies such as dose adjustment and integration with complementary treatments have been proposed to mitigate energy consumption [113,117]. Nonetheless, scalability remains uncertain due to the lack of comprehensive economic assessments and standardized data on operational costs [111,112].

While electron beam hydrolysates show promise in fermentation, limited studies have directly explored this application. Although few inhibitors are formed [114,116], further validation of hydrolysate fermentability is crucial for assessing industrial applicability and exploring higher-value products. Electron beam pretreatment has demonstrated effectiveness in small-scale applications; however, its large-scale integration presents economic and technical challenges. Cost-benefit evaluations and operational efficiency assessments are necessary to determine its feasibility within multiproduct biorefineries. Additionally, leveraging by-products such as biochar could create alternative revenue streams [115]. Further research on hardwoods and soybean residues—biomass types of economic relevance, particularly in Brazil—could expand its applicability [113,115].

In conclusion, electron beam pretreatment produces high-quality hydrolysates with minimal inhibitors, making it a promising method for advancing lignocellulosic biomass utilization. Research on energy optimization, economic feasibility, and industrial-scale validation is key to unlocking its full potential and integrating it into the bioeconomy landscape.

7.7. Extrusion

Extrusion pretreatment enhances the enzymatic digestibility of lignocellulosic biomass by inducing structural modifications, such as increased surface area and reduced cellulose crystallinity. This method adapts well to various biomass types and operational conditions, making it a promising candidate for biorefinery integration. Studies demonstrate its efficacy with both non-woody and woody biomass, with glucose yields reaching up to 95% from soybean hulls [132] and ethanol yields of 55.3% from eucalyptus wood chips [123]. These findings highlight the importance of optimizing parameters like screw speed, temperature, and moisture content for maximizing sugar recovery.

Energy efficiency remains a critical concern for extrusion pretreatment, with specific energy consumption (SEC) values ranging from 20 kJ/g to 123 kJ/g, depending on operational settings [121]. High screw speeds and low moisture content enhance sugar recovery but also increase energy demands. Balancing these factors is crucial, and integrating renewable energy sources or energy recovery systems may offer potential solutions. Moreover, precise moisture control ensures efficient mechanical shear and heat effects while minimizing excessive energy use.

Extrusion also helps manage inhibitory compounds. Post-extrusion washing, as highlighted by previous studies [124,129], effectively mitigates inhibitors like HMF and organic acids, improving sugar recovery and fermentation efficiency. Extrusion generates fewer inhibitors than other pretreatment methods, especially when aggressive chemical additives are avoided, enhancing compatibility with downstream biological processes. Furthermore, extrusion is relevant for bioethanol, biogas, and biochemical production. Methane production from extruded corn cob increased by 22.3% [130], and biogas yields from grass residues improved by 18% [134], demonstrating extrusion's potential to improve anaerobic digestibility and diversify end-product outputs for biorefinery pathways.

In conclusion, extrusion pretreatment effectively improves enzymatic accessibility and supports diverse downstream applications. However, its industrial scalability depends on advancements in energy optimization and cost reduction. Future research should refine operational parameters for specific biomass types, explore energy-efficient configurations, and validate large-scale applications. Addressing these challenges will cement extrusion's role as a core technology in sustainable biorefinery development.

7.8. Pulsed Electric Field (PEF)

Pulsed electric field (PEF) pretreatment enhances biomass porosity and enzymatic digestibility by disrupting cell membranes and increasing permeability. The method utilizes high-intensity electric pulses, which reorganize the biomass structure, facilitating enzymatic hydrolysis and biogas production [145,148]. PEF has shown promise for improving sugar yields and biogas production due to its ability to enhance biomass accessibility.

However, challenges persist regarding its scalability and energy consumption. Although PEF is energy-efficient at small scales, industrial applications face limitations due to the need for large chambers, high pulse repetition rates, and increased current demands [140]. The optimization of parameters such as field intensity and treatment time is necessary to address these issues. Studies have demonstrated that optimizing field intensity could significantly improve sugar release efficiency and reduce energy consumption [145].

Despite these challenges, PEF offers unique advantages, such as minimal inhibitor formation during standalone pretreatment, making it suitable for downstream biological processes. However, combining PEF with chemical treatments or other methods could improve efficiency and scalability. Future research should focus on optimizing PEF parameters, validating large-scale performance, and exploring its integration into biorefinery systems.

In conclusion, PEF pretreatment demonstrates considerable potential as an innovative method for processing lignocellulosic biomass. However, advancing its industrial feasibility will depend on targeted research to optimize operational parameters, validate large-scale performance, and explore its integration into multiproduct biorefineries. With further development, PEF could emerge as a sustainable and efficient technology within the bioeconomy framework.

Refining and innovating pretreatment technologies to balance cost and efficiency is critical for advancing the bioeconomy. Adjusting operational parameters to handle the variability of different biomass types is vital in this process. As the bioeconomy continues to grow, physical pretreatment methods are positioned to play a central role in unlocking the potential of lignocellulosic biomass. Overcoming current technical and economic challenges will pave the way for these methods to become integral components of industrial biorefineries. Future research should focus on hybrid technologies, energy optimization, and integration into multiproduct biorefineries, ensuring these methods contribute to a more sustainable and profitable future for biomass utilization.

Author Contributions: Conceptualization, D.B.S.J., C.J.d.A., M.K. and P.H.H.d.A.; data curation, D.B.S.J.; formal analysis, D.B.S.J.; methodology, D.B.S.J.; writing—original draft preparation, D.B.S.J.; writing—review and editing, D.B.S.J., C.J.d.A., M.K. and P.H.H.d.A.; visualization, D.B.S.J.; supervision, C.J.d.A., M.K. and P.H.H.d.A.; project administration, D.B.S.J.; funding acquisition, C.J.d.A.; resources, C.J.d.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by CAPES-PROEX (Coordination for the Improvement of Higher Education Personnel—Academic Excellence Program) through scholarship funding (88887.668530/2022-00) and SHV energy (202403525) for the financial support.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author.

Acknowledgments: The authors would like to thank the Federal University of Santa Catarina (UFSC) and Graduate Program in Chemical Engineering (PósEnq) for providing institutional and technical support.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Zhou, Z.; Liu, D.; Zhao, X. Conversion of Lignocellulose to Biofuels and Chemicals via Sugar Platform: An Updated Review on Chemistry and Mechanisms of Acid Hydrolysis of Lignocellulose. *Renew. Sustain. Energy Rev.* **2021**, *146*, 111169. [[CrossRef](#)]
2. Cheng, J. *Biomass to Renewable Energy Processes Second Edition*; CRC Press: Boca Raton, FL, USA, 2017; ISBN 9781315152868.
3. Singh, N.; Singhania, R.R.; Nigam, P.S.; Dong, C.-D.; Patel, A.K.; Puri, M. Global Status of Lignocellulosic Biorefinery: Challenges and Perspectives. *Bioresour. Technol.* **2022**, *344*, 126415. [[CrossRef](#)] [[PubMed](#)]
4. Danso, B.; Ali, S.S.; Xie, R.; Sun, J. Valorisation of Wheat Straw and Bioethanol Production by a Novel Xylanase- and Cellulase-Producing *Streptomyces* Strain Isolated from the Wood-Feeding Termite, *Microcerotermes* Species. *Fuel* **2022**, *310*, 122333. [[CrossRef](#)]
5. Van Putten, R.J.; Van Der Waal, J.C.; De Jong, E.; Rasrendra, C.B.; Heeres, H.J.; De Vries, J.G. Hydroxymethylfurfural, a Versatile Platform Chemical Made from Renewable Resources. *Chem. Rev.* **2013**, *113*, 1499–1597. [[CrossRef](#)] [[PubMed](#)]

6. Questell-Santiago, Y.M.; Galkin, M.V.; Barta, K.; Luterbacher, J.S. Stabilization Strategies in Biomass Depolymerization Using Chemical Functionalization. *Nat. Rev. Chem.* **2020**, *4*, 311–330. [\[CrossRef\]](#)
7. Sharma, S.; Tsai, M.L.; Sharma, V.; Sun, P.P.; Nargotra, P.; Bajaj, B.K.; Chen, C.W.; Dong, C. Di Environment Friendly Pretreatment Approaches for the Bioconversion of Lignocellulosic Biomass into Biofuels and Value-Added Products. *Environments* **2023**, *10*, 6. [\[CrossRef\]](#)
8. Wang, C.; Zhang, X.; Liu, Q.; Zhang, Q.; Chen, L.; Ma, L. A Review of Conversion of Lignocellulose Biomass to Liquid Transport Fuels by Integrated Refining Strategies. *Fuel Process. Technol.* **2020**, *208*, 106485. [\[CrossRef\]](#)
9. Haldar, D.; Purkait, M.K. A Review on the Environment-Friendly Emerging Techniques for Pretreatment of Lignocellulosic Biomass: Mechanistic Insight and Advancements. *Chemosphere* **2021**, *264*, 128523. [\[CrossRef\]](#)
10. Saini, J.K.; Himanshu; Hemansi; Kaur, A.; Mathur, A. Strategies to Enhance Enzymatic Hydrolysis of Lignocellulosic Biomass for Biorefinery Applications: A Review. *Bioresour. Technol.* **2022**, *360*, 127517. [\[CrossRef\]](#)
11. Guo, H.; Chang, Y.; Lee, D.J. Enzymatic Saccharification of Lignocellulosic Biorefinery: Research Focuses. *Bioresour. Technol.* **2018**, *252*, 198–215. [\[CrossRef\]](#)
12. Mosier, N.; Wyman, C.; Dale, B.; Elander, R.; Lee, Y.Y.; Holtzapple, M.; Ladisch, M. Features of Promising Technologies for Pretreatment of Lignocellulosic Biomass. *Bioresour. Technol.* **2005**, *96*, 673–686. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Mankar, A.R.; Pandey, A.; Modak, A.; Pant, K.K. Pretreatment of Lignocellulosic Biomass: A Review on Recent Advances. *Bioresour. Technol.* **2021**, *334*, 125235. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Yousuf, A.; Sannino, F.; Pirozzi, D. *Lignocellulosic Biomass to Liquid Biofuels*; Academic Press: Cambridge, MA, USA, 2019; ISBN 978-0-12-815936-1.
15. Agbor, V.B.; Cicek, N.; Sparling, R.; Berlin, A.; Levin, D.B. Biomass Pretreatment: Fundamentals toward Application. *Biotechnol. Adv.* **2011**, *29*, 675–685. [\[CrossRef\]](#)
16. Zheng, Y.; Shi, J.; Tu, M.; Cheng, Y.S. Principles and Development of Lignocellulosic Biomass Pretreatment for Biofuels. *Adv. Bioenergy* **2017**, *2*, 1–68. [\[CrossRef\]](#)
17. Gupta, A.; Verma, J.P. Sustainable Bio-Ethanol Production from Agro-Residues: A Review. *Renew. Sustain. Energy Rev.* **2015**, *41*, 550–567. [\[CrossRef\]](#)
18. Rezaia, S.; Oryani, B.; Cho, J.; Talaiekhosani, A.; Sabbagh, F.; Hashemi, B.; Rupani, P.F.; Mohammadi, A.A. Different Pretreatment Technologies of Lignocellulosic Biomass for Bioethanol Production: An Overview. *Energy* **2020**, *199*, 117457. [\[CrossRef\]](#)
19. Hassan, S.S.; Williams, G.A.; Jaiswal, A.K. Emerging Technologies for the Pretreatment of Lignocellulosic Biomass. *Bioresour. Technol.* **2018**, *262*, 310–318. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Himmel, M.E.; Ding, S.Y.; Johnson, D.K.; Adney, W.S.; Nimlos, M.R.; Brady, J.W.; Foust, T.D. Biomass Recalcitrance: Engineering Plants and Enzymes for Biofuels Production. *Science* **2007**, *315*, 804–807. [\[CrossRef\]](#) [\[PubMed\]](#)
21. Harmsen, P.F.H.; Huijgen, W.; Bermudez, L.; Bakker, R. *Literature Review of Physical and Chemical Pretreatment Processes for Lignocellulosic Biomass*; Wageningen UR Food & Biobased Research: Wageningen, The Netherlands, 2010; ISBN 9789085857570.
22. Chen, S.; Zhang, X.; Singh, D.; Yu, H.; Yang, X. Biological Pretreatment of Lignocellulosics: Potential, Progress and Challenges. *Biofuels* **2010**, *1*, 177–199. [\[CrossRef\]](#)
23. Taherzadeh, M.J.; Karimi, K. Pretreatment of Lignocellulosic Wastes to Improve Ethanol and Biogas Production: A Review. *Int. J. Mol. Sci.* **2008**, *9*, 1621–1651. [\[CrossRef\]](#)
24. Ashokkumar, V.; Venkatkarthick, R.; Jayashree, S.; Chuetor, S.; Dharmaraj, S.; Kumar, G.; Chen, W.H.; Ngamcharussrivichai, C. Recent Advances in Lignocellulosic Biomass for Biofuels and Value-Added Bioproducts—A Critical Review. *Bioresour. Technol.* **2022**, *344*, 126195. [\[CrossRef\]](#)
25. Demirbas, A. Biofuels from Agricultural Biomass. *Energy Sources Part A Recovery Util. Environ. Eff.* **2009**, *31*, 1573–1582. [\[CrossRef\]](#)
26. Kumar, R.; Singh, S.; Singh, O.V. Bioconversion of Lignocellulosic Biomass: Biochemical and Molecular Perspectives. *J. Ind. Microbiol. Biotechnol.* **2008**, *35*, 377–391. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Kassaye, S.; Pant, K.K.; Jain, S. Synergistic Effect of Ionic Liquid and Dilute Sulphuric Acid in the Hydrolysis of Microcrystalline Cellulose. *Fuel Process. Technol.* **2016**, *148*, 289–294. [\[CrossRef\]](#)
28. Lee, H.V.; Hamid, S.B.A.; Zain, S.K. Conversion of Lignocellulosic Biomass to Nanocellulose: Structure and Chemical Process. *Sci. World J.* **2014**, *2014*, 631013. [\[CrossRef\]](#)
29. Singhvi, M.S.; Gokhale, D.V. Lignocellulosic Biomass: Hurdles and Challenges in Its Valorization. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 9305–9320. [\[CrossRef\]](#)
30. Velvizhi, G.; Balakumar, K.; Shetti, N.P.; Ahmad, E.; Kishore Pant, K.; Aminabhavi, T.M. Integrated Biorefinery Processes for Conversion of Lignocellulosic Biomass to Value Added Materials: Paving a Path towards Circular Economy. *Bioresour. Technol.* **2022**, *343*, 126151. [\[CrossRef\]](#)
31. Dora, S.; Bhaskar, T.; Singh, R.; Naik, D.V.; Adhikari, D.K. Effective Catalytic Conversion of Cellulose into High Yields of Methyl Glucosides over Sulfonated Carbon Based Catalyst. *Bioresour. Technol.* **2012**, *120*, 318–321. [\[CrossRef\]](#)

32. Zang, G.; Shah, A.; Wan, C. Techno-Economic Analysis of an Integrated Biorefinery Strategy Based on One-Pot Biomass Fractionation and Furfural Production. *J. Clean. Prod.* **2020**, *260*, 120837. [CrossRef]
33. Wang, F.; Ouyang, D.; Zhou, Z.; Page, S.J.; Liu, D.; Zhao, X. Lignocellulosic Biomass as Sustainable Feedstock and Materials for Power Generation and Energy Storage. *J. Energy Chem.* **2021**, *57*, 247–280. [CrossRef]
34. IBGE—Instituto Brasileiro de Geografia e Estatística. Produção Agropecuária. Available online: <https://www.ibge.gov.br/explica/producao-agropecuaria/> (accessed on 5 March 2024).
35. Jędrzejczyk, M.; Soszka, E.; Czapnik, M.; Ruppert, A.M.; Grams, J. Physical and Chemical Pretreatment of Lignocellulosic Biomass. In *Second and Third Generation of Feedstocks: The Evolution of Biofuels*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 143–196. [CrossRef]
36. Shah, A.A.; Seehar, T.H.; Sharma, K.; Toor, S.S. Biomass Pretreatment Technologies. In *Hydrocarbon Biorefinery: Sustainable Processing of Biomass for Hydrocarbon Biofuels*; Elsevier: Amsterdam, The Netherlands, 2022; pp. 203–228. [CrossRef]
37. Moodley, P.; Trois, C. Lignocellulosic Biorefineries: The Path Forward. In *Sustainable Biofuels: Opportunities and Challenges*; Academic Press: Cambridge, MA, USA, 2021; pp. 21–42. [CrossRef]
38. Kumar, P.; Barrett, D.M.; Delwiche, M.J.; Stroeve, P. Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production. *Ind. Eng. Chem. Res.* **2009**, *48*, 3713–3729. [CrossRef]
39. Zheng, Y.; Zhao, J.; Xu, F.; Li, Y. Pretreatment of Lignocellulosic Biomass for Enhanced Biogas Production. *Prog. Energy Combust. Sci.* **2014**, *42*, 35–53. [CrossRef]
40. Buaban, B.; Inoue, H.; Yano, S.; Tanapongpipat, S.; Ruanglek, V.; Champreda, V.; Pichyangkura, R.; Rengpipat, S.; Eurwilaichitr, L. Bioethanol Production from Ball Milled Bagasse Using an On-Site Produced Fungal Enzyme Cocktail and Xylose-Fermenting *Pichia Stipitis*. *J. Biosci. Bioeng.* **2010**, *110*, 18–25. [CrossRef] [PubMed]
41. Da Silva, A.S.A.; Inoue, H.; Endo, T.; Yano, S.; Bon, E.P.S. Milling Pretreatment of Sugarcane Bagasse and Straw for Enzymatic Hydrolysis and Ethanol Fermentation. *Bioresour. Technol.* **2010**, *101*, 7402–7409. [CrossRef]
42. Zeng, M.; Mosier, N.S.; Huang, C.P.; Sherman, D.M.; Ladisch, M.R. Microscopic Examination of Changes of Plant Cell Structure in Corn Stover Due to Hot Water Pretreatment and Enzymatic Hydrolysis. *Biotechnol. Bioeng.* **2007**, *97*, 265–278. [CrossRef]
43. Zhu, J.Y.; Pan, X.; Zalesny, R.S. Pretreatment of Woody Biomass for Biofuel Production: Energy Efficiency, Technologies, and Recalcitrance. *Appl. Microbiol. Biotechnol.* **2010**, *87*, 847–857. [CrossRef] [PubMed]
44. Inoue, H.; Yano, S.; Endo, T.; Sakaki, T.; Sawayama, S. Combining Hot-Compressed Water and Ball Milling Pretreatments to Improve the Efficiency of the Enzymatic Hydrolysis of Eucalyptus. *Biotechnol. Biofuels* **2008**, *1*, 2. [CrossRef]
45. Al-Assaf, S.; Phillips, G.O.; Williams, P.A.; du Plessis, T.A. Application of Ionizing Radiations to Produce New Polysaccharides and Proteins with Enhanced Functionality. *Nucl. Instruments Methods Phys. Res. Sect. B Beam Interact. Mater. Atoms* **2007**, *265*, 37–43. [CrossRef]
46. Kassim, M.A.; Khalil, H.P.A.; Serri, N.A.; Kassim, M.H.M.; Syakir, M.I.; Aprila, N.A.S.; Dungani, R.; Kassim, M.A.; Khalil, H.P.A.; Serri, N.A.; et al. Irradiation Pretreatment of Tropical Biomass and Biofiber for Biofuel Production. In *Radiation Effects in Materials*; IntechOpen: London, UK, 2016. [CrossRef]
47. Mason, T.J.; Lorimer, J.P. *Applied Sonochemistry*; Wiley: Hoboken, NJ, USA, 2002. [CrossRef]
48. Bussemaker, M.J.; Zhang, D. Effect of Ultrasound on Lignocellulosic Biomass as a Pretreatment for Biorefinery and Biofuel Applications. *Ind. Eng. Chem. Res.* **2013**, *52*, 3563–3580. [CrossRef]
49. Zuo, M.; Xiao, R.; Du, F.; Cheng, C.; Rodriguez, R.D.; Ma, L.; Zhu, B.; Qiu, L. Ultrasound-Activated Mechanochemical Reactions for Controllable Biomedical Applications. *Smart Mater. Med.* **2024**, *5*, 461–476. [CrossRef]
50. Zhu, X.; Das, R.S.; Bhavya, M.L.; Garcia-Vaquero, M.; Tiwari, B.K. Acoustic Cavitation for Agri-Food Applications: Mechanism of Action, Design of New Systems, Challenges and Strategies for Scale-Up. *Ultrason. Sonochem.* **2024**, *105*, 106850. [CrossRef] [PubMed]
51. Kunaver, M.; Jasiukaityte, E.; Čuk, N. Ultrasonically Assisted Liquefaction of Lignocellulosic Materials. *Bioresour. Technol.* **2012**, *103*, 360–366. [CrossRef]
52. Ravindran, R.; Jaiswal, A.K. A Comprehensive Review on Pre-Treatment Strategy for Lignocellulosic Food Industry Waste: Challenges and Opportunities. *Bioresour. Technol.* **2016**, *199*, 92–102. [CrossRef]
53. Manickam, S.; Camilla Boffito, D.; Flores, E.M.M.; Leveque, J.M.; Pflieger, R.; Pollet, B.G.; Ashokkumar, M. Ultrasonics and Sonochemistry: Editors’ Perspective. *Ultrason. Sonochem.* **2023**, *99*, 106540. [CrossRef]
54. Liu, H.; Zhang, Y.; Yang, L.; Wu, Z. Effects of Ultrasound Pretreatment on Microstructure and Drying Characteristics of Eucalyptus Urophylla × E. Grandis. *BioResources* **2018**, *13*, 5953–5964. [CrossRef]
55. He, Z.; Qian, J.; Wang, Z.; Yi, S.; Mu, J. Effects of Ultrasound Pretreatment on Eucalyptus Thermal Decomposition Characteristics As Determined by Thermogravimetric, Differential Scanning Calorimetry, and Fourier Transform Infrared Analysis. *ACS Omega* **2018**, *3*, 6611–6616. [CrossRef] [PubMed]
56. He, Z.; Wang, Z.; Zhao, Z.; Yi, S.; Mu, J.; Wang, X. Influence of Ultrasound Pretreatment on Wood Physiochemical Structure. *Ultrason. Sonochem.* **2017**, *34*, 136–141. [CrossRef]

57. Revin, V.; Atykyan, N.; Zakharkin, D. Enzymatic Hydrolysis and Fermentation of Ultradispersed Wood Particles after Ultrasonic Pretreatment. *Electron. J. Biotechnol.* **2016**, *20*, 14–19. [\[CrossRef\]](#)
58. Sharma, V.; Nargotra, P.; Sharma, S.; Bajaj, B.K. Efficacy and Functional Mechanisms of a Novel Combinatorial Pretreatment Approach Based on Deep Eutectic Solvent and Ultrasonic Waves for Bioconversion of Sugarcane Bagasse. *Renew. Energy* **2021**, *163*, 1910–1922. [\[CrossRef\]](#)
59. Candido, R.G.; Mori, N.R.; Gonçalves, A.R. Sugarcane Straw as Feedstock for 2G Ethanol: Evaluation of Pretreatments and Enzymatic Hydrolysis. *Ind. Crop. Prod.* **2019**, *142*, 111845. [\[CrossRef\]](#)
60. Martinez-Jimenez, F.D.; Macie Pinto, M.P.; Mudhoo, A.; De Alencar Neves, T.; Rostagno, M.A.; Forster-Carneiro, T. Influence of Ultrasound Irradiation Pre-Treatment in Biohythane Generation from the Thermophilic Anaerobic Co-Digestion of Sugar Production Residues. *J. Environ. Chem. Eng.* **2017**, *5*, 3749–3758. [\[CrossRef\]](#)
61. Eblaghi, M.; Niakousari, M.; Sarshar, M.; Mesbahi, G.R. Combining Ultrasound with Mild Alkaline Solutions as an Effective Pretreatment to Boost the Release of Sugar Trapped in Sugarcane Bagasse for Bioethanol Production. *J. Food Process Eng.* **2016**, *39*, 273–282. [\[CrossRef\]](#)
62. Liu, C.F.; Sun, R.C.; Qin, M.H.; Zhang, A.P.; Ren, J.L.; Xu, F.; Ye, J.; Wu, S. Bin Chemical Modification of Ultrasound-Pretreated Sugarcane Bagasse with Maleic Anhydride. *Ind. Crop. Prod.* **2007**, *26*, 212–219. [\[CrossRef\]](#)
63. Velmurugan, R.; Muthukumar, K. Utilization of Sugarcane Bagasse for Bioethanol Production: Sono-Assisted Acid Hydrolysis Approach. *Bioresour. Technol.* **2011**, *102*, 7119–7123. [\[CrossRef\]](#) [\[PubMed\]](#)
64. Velmurugan, R.; Muthukumar, K. Sono-Assisted Enzymatic Saccharification of Sugarcane Bagasse for Bioethanol Production. *Biochem. Eng. J.* **2012**, *63*, 1–9. [\[CrossRef\]](#)
65. Ramadoss, G.; Muthukumar, K. Ultrasound Assisted Ammonia Pretreatment of Sugarcane Bagasse for Fermentable Sugar Production. *Biochem. Eng. J.* **2014**, *83*, 33–41. [\[CrossRef\]](#)
66. Li, Q.; Ji, G.S.; Tang, Y.B.; Gu, X.D.; Fei, J.J.; Jiang, H.Q. Ultrasound-Assisted Compatible in Situ Hydrolysis of Sugarcane Bagasse in Cellulase-Aqueous-N-Methylmorpholine-N-Oxide System for Improved Saccharification. *Bioresour. Technol.* **2012**, *107*, 251–257. [\[CrossRef\]](#)
67. Esfahani, M.R.; Azin, M. Pretreatment of Sugarcane Bagasse by Ultrasound Energy and Dilute Acid. *Asia-Pac. J. Chem. Eng.* **2012**, *7*, 274–278. [\[CrossRef\]](#)
68. Velmurugan, R.; Muthukumar, K. Ultrasound-Assisted Alkaline Pretreatment of Sugarcane Bagasse for Fermentable Sugar Production: Optimization through Response Surface Methodology. *Bioresour. Technol.* **2012**, *112*, 293–299. [\[CrossRef\]](#)
69. Sun, J.X.; Xu, F.; Sun, X.F.; Sun, R.C.; Wu, S.B. Comparative Study of Lignins from Ultrasonic Irradiated Sugar-Cane Bagasse. *Polym. Int.* **2004**, *53*, 1711–1721. [\[CrossRef\]](#)
70. Pérez-Rodríguez, N.; García-Bernet, D.; Domínguez, J.M. Effects of Enzymatic Hydrolysis and Ultrasounds Pretreatments on Corn Cob and Vine Trimming Shoots for Biogas Production. *Bioresour. Technol.* **2016**, *221*, 130–138. [\[CrossRef\]](#) [\[PubMed\]](#)
71. Yin, J.; Hao, L.; Yu, W.; Wang, E.; Zhao, M.; Xu, Q.; Liu, Y. Enzymatic Hydrolysis Enhancement of Corn Lignocellulose by Supercritical CO₂ Combined with Ultrasound Pretreatment. *Chin. J. Catal.* **2014**, *35*, 763–769. [\[CrossRef\]](#)
72. Hu, X.; Xiao, Y.; Niu, K.; Zhao, Y.; Zhang, B.; Hu, B. Functional Ionic Liquids for Hydrolysis of Lignocellulose. *Carbohydr. Polym.* **2013**, *97*, 172–176. [\[CrossRef\]](#)
73. Liu, J.H.; Wang, F.M. Study on Technology of Reducing Sugar Production from Bermuda Grass by Combination of Dilute Acid and Ultrasound. *Biotechnology* **2015**, *14*, 274–278. [\[CrossRef\]](#)
74. Leonelli, C.; Mason, T.J. Microwave and Ultrasonic Processing: Now a Realistic Option for Industry. *Chem. Eng. Process. Process Intensif.* **2010**, *49*, 885–900. [\[CrossRef\]](#)
75. Pellerá, F.M.; Gidarakos, E. Microwave Pretreatment of Lignocellulosic Agroindustrial Waste for Methane Production. *J. Environ. Chem. Eng.* **2017**, *5*, 352–365. [\[CrossRef\]](#)
76. Angoy, A.; Brianceau, S.; Chabrier, F.; Ginisty, P.; Jomaa, W.; Rochas, J.F.; Sommer, A.; Valat, M. Microwave Technology for Food Applications. In *Green Food Processing Techniques: Preservation, Transformation and Extraction*; Academic Press: Cambridge, MA, USA, 2019; pp. 455–498. [\[CrossRef\]](#)
77. Caballero, B.; Trugo, L.; Finglas, P. *Encyclopedia of Food Sciences and Nutrition*, 2nd ed.; Caballero, B., Finglas, P., Toldra, F., Eds.; Academic Press: Cambridge, MA, USA, 2003; ISBN 9780080917917.
78. Hou, A.; Wang, X.; Wu, L. Effect of Microwave Irradiation on the Physical Properties and Morphological Structures of Cotton Cellulose. *Carbohydr. Polym.* **2008**, *74*, 934–937. [\[CrossRef\]](#)
79. Keshwani, D.R.; Cheng, J.J. Microwave-Based Alkali Pretreatment of Switchgrass and Coastal Bermudagrass for Bioethanol Production. *Biotechnol. Prog.* **2010**, *26*, 644–652. [\[CrossRef\]](#)
80. Aguilar-Reynosa, A.; Román, A.; Rodríguez-Jasso, R.M.; Aguilar, C.N.; Garrote, G.; Ruiz, H.A. Comparison of Microwave and Conduction-Convection Heating Autohydrolysis Pretreatment for Bioethanol Production. *Bioresour. Technol.* **2017**, *243*, 273–283. [\[CrossRef\]](#)

81. Hu, Z.; Wen, Z. Enhancing Enzymatic Digestibility of Switchgrass by Microwave-Assisted Alkali Pretreatment. *Biochem. Eng. J.* **2008**, *38*, 369–378. [\[CrossRef\]](#)
82. Kostas, E.T.; Beneroso, D.; Robinson, J.P. The Application of Microwave Heating in Bioenergy: A Review on the Microwave Pre-Treatment and Upgrading Technologies for Biomass. *Renew. Sustain. Energy Rev.* **2017**, *77*, 12–27. [\[CrossRef\]](#)
83. Amini, N.; Haritos, V.S.; Tanksale, A. Microwave Assisted Pretreatment of Eucalyptus Sawdust Enhances Enzymatic Saccharification and Maximizes Fermentable Sugar Yield. *Renew. Energy* **2018**, *127*, 653–660. [\[CrossRef\]](#)
84. Bichot, A.; Lerosty, M.; Méchin, V.; Bernet, N.; Delgenès, J.P.; García-Bernet, D. Evaluation of Chemical-Free Microwave Pretreatment on Methane Yield of Two Grass Biomass with Contrasted Parietal Content. *Energy Convers. Manag.* **2021**, *229*, 113746. [\[CrossRef\]](#)
85. Binod, P.; Satyanagalakshmi, K.; Sindhu, R.; Janu, K.U.; Sukumaran, R.K.; Pandey, A. Short Duration Microwave Assisted Pretreatment Enhances the Enzymatic Saccharification and Fermentable Sugar Yield from Sugarcane Bagasse. *Renew. Energy* **2012**, *37*, 109–116. [\[CrossRef\]](#)
86. Boonsombuti, A.; Wanapirom, R.; Luengnaruemitchai, A.; Wongkasemjit, S. The Effect of the Addition of Acetic Acid to Aqueous Ionic Liquid Mixture Using Microwave-Assisted Pretreatment in the Saccharification of Napier Grass. *Waste Biomass Valorization* **2018**, *9*, 1795–1804. [\[CrossRef\]](#)
87. Fonseca, B.C.; Reginatto, V.; López-Linares, J.C.; Lucas, S.; García-Cubero, M.T.; Coca, M. Ideal Conditions of Microwave-Assisted Acid Pretreatment of Sugarcane Straw Allow Fermentative Butyric Acid Production without Detoxification Step. *Bioresour. Technol.* **2021**, *329*, 124929. [\[CrossRef\]](#)
88. Hermiati, E.; Pramasari, D.A.; Ermawar, R.A.; Sondari, D.; Lianawati, A.; Sudiyani, Y.; Adilina, I.B. Microwave Pretreatment of Sugarcane Trash and Oil Palm Empty Fruit Bunch with an Aluminum Sulfate Catalyst for Improvement of Sugar Recovery. *Waste Biomass Valorization* **2024**, *15*, 437–453. [\[CrossRef\]](#)
89. Irmak, S.; Meryemoglu, B.; Sandip, A.; Subbiah, J.; Mitchell, R.B.; Sarath, G. Microwave Pretreatment Effects on Switchgrass and Miscanthus Solubilization in Subcritical Water and Hydrolysate Utilization for Hydrogen Production. *Biomass Bioenergy* **2018**, *108*, 48–54. [\[CrossRef\]](#)
90. Karunanithy, C.; Muthukumarappan, K.; Gibbons, W.R. Sequential Extrusion-Microwave Pretreatment of Switchgrass and Big Bluestem. *Bioresour. Technol.* **2014**, *153*, 393–398. [\[CrossRef\]](#)
91. Kłosowski, G.; Mikulski, D.; Lewandowska, N. Microwave-Assisted Degradation of Biomass with the Use of Acid Catalysis. *Catalysts* **2020**, *10*, 641. [\[CrossRef\]](#)
92. Miranda, I.; Masiero, M.O.; Zama, T.; Capella, M.; Lalue, C. Improved Pretreatments Applied to the Sugarcane Bagasse and Release of Lignin and Hemicellulose from the Cellulose-Enriched Fractions by Sulfuric Acid Hydrolysis. *J. Chem. Technol. Biotechnol.* **2016**, *91*, 476–482. [\[CrossRef\]](#)
93. Moodley, P.; Gueguim Kana, E.B. Bioethanol Production from Sugarcane Leaf Waste: Effect of Various Optimized Pretreatments and Fermentation Conditions on Process Kinetics. *Biotechnol. Rep.* **2019**, *22*, e00329. [\[CrossRef\]](#) [\[PubMed\]](#)
94. Han, M.X.; Li, D.M.; Feng, Y.J.; Tan, Y.F.; Yuan, Y.X. Microwave Pre-Treatment of Corn Straw and Its Enzymatic Hydrolysis. *Adv. Mater. Res.* **2013**, *641–642*, 971–974. [\[CrossRef\]](#)
95. Rigual, V.; Domínguez, J.C.; Santos, T.M.; Rivas, S.; Alonso, M.V.; Oliet, M.; Rodriguez, F. Autohydrolysis and Microwave Ionic Liquid Pretreatment of Pinus Radiata: Imaging Visualization and Analysis to Understand Enzymatic Digestibility. *Ind. Crops Prod.* **2019**, *134*, 328–337. [\[CrossRef\]](#)
96. Rigual, V.; Santos, T.M.; Domínguez, J.C.; Alonso, M.V.; Oliet, M.; Rodriguez, F. Evaluation of Hardwood and Softwood Fractionation Using Autohydrolysis and Ionic Liquid Microwave Pretreatment. *Biomass Bioenergy* **2018**, *117*, 190–197. [\[CrossRef\]](#)
97. Zhu, Z.; Rezende, C.A.; Simister, R.; McQueen-Mason, S.J.; Macquarrie, D.J.; Polikarpov, I.; Gomez, L.D. Efficient Sugar Production from Sugarcane Bagasse by Microwave Assisted Acid and Alkali Pretreatment. *Biomass Bioenergy* **2016**, *93*, 269–278. [\[CrossRef\]](#)
98. Sasaki, C.; Takada, R.; Watanabe, T.; Honda, Y.; Karita, S.; Nakamura, Y.; Watanabe, T. Surface Carbohydrate Analysis and Bioethanol Production of Sugarcane Bagasse Pretreated with the White Rot Fungus, *Ceriporiopsis subvermispora* and Microwave Hydrothermolysis. *Bioresour. Technol.* **2011**, *102*, 9942–9946. [\[CrossRef\]](#)
99. Wang, C.; Shao, Z.; Qiu, L.; Hao, W.; Qu, Q.; Sun, G. The Solid-State Physicochemical Properties and Biogas Production of the Anaerobic Digestion of Corn Straw Pretreated by Microwave Irradiation. *RSC Adv.* **2021**, *11*, 3575–3584. [\[CrossRef\]](#)
100. Ude, M.U.; Oluka, I. Optimization of Microwave Pretreatment Parameters of Elephant Grass by Response Surface Methodology and Kinetic Study of Its Biomethanation. *Biofuels* **2022**, *13*, 863–872. [\[CrossRef\]](#)
101. Fan, X.; Niemira, B.A. Gamma Ray, Electron Beam, and X-Ray Irradiation. In *Food Safety Engineering*; Food Engineering Series; Springer: Cham, Switzerland, 2020; pp. 471–492. [\[CrossRef\]](#)
102. Liu, Y.; Chen, J.; Wu, X.; Wang, K.; Su, X.; Chen, L.; Zhou, H.; Xiong, X. Insights into the Effects of γ -Irradiation on the Microstructure, Thermal Stability and Irradiation-Derived Degradation Components of Microcrystalline Cellulose (MCC). *RSC Adv.* **2015**, *5*, 34353–34363. [\[CrossRef\]](#)

103. Kumar, A.; Kumar, G.A. Modification of Lignin Properties Using Alpha Particles and Gamma-Rays for Diverse Applications. *Radiat. Phys. Chem.* **2023**, *202*, 110562. [CrossRef]
104. Barbara, P.F. Research Needs and Opportunities in Radiation Chemistry Workshop. 1998. Available online: <https://www.osti.gov/biblio/1302247> (accessed on 9 March 2024).
105. Al Gharib, S.; Mostafavi, M.; Belloni, J. Radiation-Assisted Hydrolysis of Lignocellulosic Biomass. Mechanistic Study. *Waste Biomass Valorization* **2023**, *14*, 1113–1122. [CrossRef]
106. Kapoor, K.; Tyagi, A.K.; Das, M.; Kumar, V. Comparative Analysis of Morphological and Structural Changes in Gamma and Electron Beam Irradiated Sugarcane Bagasse. *Cellul. Chem. Technol.* **2023**, *57*, 61–70. [CrossRef]
107. Wu, X.; Chen, L.; He, W.; Qi, H.; Zhang, Y.; Zhou, Y.; Su, X.; Deng, M.; Wang, K. Characterize the Physicochemical Structure and Enzymatic Efficiency of Agricultural Residues Exposed to γ -Irradiation Pretreatment. *Ind. Crop. Prod.* **2020**, *150*, 112228. [CrossRef]
108. Li, Q.-M.; Li, X.-J.; Jiang, Y.-L.; Xiong, X.-Y.; Hu, Q.-L.; Tan, X.-H.; Wang, K.-Q.; Su, X.-J. Analysis of Degradation Products and Structural Characterization of Giant Reed and Chinese Silvergrass Pretreated by ^{60}Co - γ Irradiation. *Ind. Crop. Prod.* **2016**, *83*, 307–315. [CrossRef]
109. Bak, J.S.; Ko, J.K.; Han, Y.H.; Lee, B.C.; Choi, I.G.; Kim, K.H. Improved Enzymatic Hydrolysis Yield of Rice Straw Using Electron Beam Irradiation Pretreatment. *Bioresour. Technol.* **2009**, *100*, 1285–1290. [CrossRef]
110. Singh, S.V.; Devanandhan, S.; Lakhina, G.S.; Bharuthram, R. Electron Acoustic Solitary Waves in a Magnetized Plasma with Nonthermal Electrons and an Electron Beam. *Phys. Plasmas* **2016**, *23*, 82310. [CrossRef]
111. Jusri, N.A.A.; Azizan, A.; Zain, Z.S.Z.; Rahman, M.F.A. Effect of Electron Beam Irradiation and Ionic Liquid Combined Pretreatment Method on Various Lignocellulosic Biomass. *Key Eng. Mater.* **2019**, *797*, 351–358. [CrossRef]
112. Siwek, M.; Edgecock, T. Application of Electron Beam Radiolysis for Sewage Sludge Treatment—A Review. *Environ. Sci. Pollut. Res.* **2020**, *27*, 42424–42448. [CrossRef]
113. Duarte, C.L.; Ribeiro, M.A.; Oikawa, H.; Mori, M.N.; Napolitano, C.M.; Galvão, C.A. Electron Beam Combined with Hydrothermal Treatment for Enhancing the Enzymatic Convertibility of Sugarcane Bagasse. *Radiat. Phys. Chem.* **2012**, *81*, 1008–1011. [CrossRef]
114. Guo, X.; Shu, S.; Zhang, W.; Wang, E.; Hao, J. Synergetic Degradation of Corn Cob with Inorganic Salt (or Hydrogen Peroxide) and Electron Beam Irradiation. *ACS Sustain. Chem. Eng.* **2016**, *4*, 1099–1105. [CrossRef]
115. Rattanawongwiboon, T.; Chanklinhorm, P.; Chutimasakul, T.; Kwamman, T.; Kingkam, W.; Khamlue, R.; Ummartyotin, S. Green Acidic Catalyst from Cellulose Extracted from Sugarcane Bagasse through Pretreatment by Electron Beam Irradiation and Subsequent Sulfonation for Sugar Production. *J. Met. Mater. Miner.* **2022**, *32*, 134–142. [CrossRef]
116. Karthika, K.; Arun, A.B.; Rekha, P.D. Enzymatic Hydrolysis and Characterization of Lignocellulosic Biomass Exposed to Electron Beam Irradiation. *Carbohydr. Polym.* **2012**, *90*, 1038–1045. [CrossRef] [PubMed]
117. Shen, H.; Yu, J.; Bai, J.; Ge, X.; Liang, W.; Ospankulova, G.; Muratkhan, M.; Zhang, G.; Li, W. Electron Beam Irradiation Regulates the Structure and Functionality of Ball-Milled Corn Starch: The Related Mechanism. *Carbohydr. Polym.* **2022**, *297*, 120016. [CrossRef]
118. Duque, A.; Manzanares, P.; Ballesteros, M. Extrusion as a Pretreatment for Lignocellulosic Biomass: Fundamentals and Applications. *Renew. Energy* **2017**, *114*, 1427–1441. [CrossRef]
119. Zheng, J.; Rehmann, L. Extrusion Pretreatment of Lignocellulosic Biomass: A Review. *Int. J. Mol. Sci.* **2014**, *15*, 18967–18984. [CrossRef]
120. Konan, D.; Koffi, E.; Ndao, A.; Peterson, E.C.; Rodrigue, D.; Feng, W.; Konan, D.; Koffi, E.; Ndao, A.; Peterson, E.C.; et al. An Overview of Extrusion as a Pretreatment Method of Lignocellulosic Biomass. *Energies* **2022**, *15*, 3002. [CrossRef]
121. Kuster Moro, M.; Sposina Sobral Teixeira, R.; Sant’Ana da Silva, A.; Duarte Fujimoto, M.; Albuquerque Melo, P.; Resende Secchi, A.; Pinto da Silva Bon, E. Continuous Pretreatment of Sugarcane Biomass Using a Twin-Screw Extruder. *Ind. Crop. Prod.* **2017**, *97*, 509–517. [CrossRef]
122. Doménech, P.; Manzanares, P.; Álvarez, C.; Ballesteros, M.; Duque, A. Comprehensive Study on the Effects of Process Parameters of Alkaline Thermal Pretreatment Followed by Thermomechanical Extrusion in Sugar Liberation from Eucalyptus Grandis Wood. *Holzforchung* **2021**, *75*, 250–259. [CrossRef]
123. Tian, D.; Shen, F.; Yang, G.; Deng, S.; Long, L.; He, J.; Zhang, J.; Huang, C.; Luo, L. Liquid Hot Water Extraction Followed by Mechanical Extrusion as a Chemical-Free Pretreatment Approach for Cellulosic Ethanol Production from Rigid Hardwood. *Fuel* **2019**, *252*, 589–597. [CrossRef]
124. Duque, A.; Manzanares, P.; González, A.; Ballesteros, M. Study of the Application of Alkaline Extrusion to the Pretreatment of Eucalyptus Biomass as First Step in a Bioethanol Production Process. *Energies* **2018**, *11*, 2961. [CrossRef]
125. Liang, Y.; Lei, B.; Zhong, H.T.; Feng, Y.H.; Qu, J.P. A Promising Screw-Extrusion Steam Explosion Pretreatment Process: Effects on the Morphological and Structural Features of Eucalyptus Woodchips. *RSC Adv.* **2016**, *6*, 109657–109663. [CrossRef]
126. Lee, S.H.; Inoue, S.; Teramoto, Y.; Endo, T. Enzymatic Saccharification of Woody Biomass Micro/Nanofibrillated by Continuous Extrusion Process II: Effect of Hot-Compressed Water Treatment. *Bioresour. Technol.* **2010**, *101*, 9645–9649. [CrossRef]

127. Karunanithy, C.; Muthukumarappan, K.; Gibbons, W.R. Extrusion Pretreatment of Pine Wood Chips. *Appl. Biochem. Biotechnol.* **2012**, *167*, 81–99. [\[CrossRef\]](#)
128. Fasheun, D.O.; de Oliveira, R.A.; Bon, E.P.S.; da Silva, A.S.A.; Teixeira, R.S.S.; Ferreira-Leitão, V.S. Dry Extrusion Pretreatment of Cassava Starch Aided by Sugarcane Bagasse for Improved Starch Saccharification. *Carbohydr. Polym.* **2022**, *285*, 119256. [\[CrossRef\]](#)
129. Da Silva, A.S.A.; Teixeira, R.S.S.; Endo, T.; Bon, E.P.S.; Lee, S.H. Continuous Pretreatment of Sugarcane Bagasse at High Loading in an Ionic Liquid Using a Twin-Screw Extruder. *Green Chem.* **2013**, *15*, 1991–2001. [\[CrossRef\]](#)
130. Pérez-Rodríguez, N.; García-Bernet, D.; Domínguez, J.M. Extrusion and Enzymatic Hydrolysis as Pretreatments on Corn Cob for Biogas Production. *Renew. Energy* **2017**, *107*, 597–603. [\[CrossRef\]](#)
131. Yoo, J.; Alavi, S.; Vadlani, P.; Behnke, K.C. Soybean Hulls Pretreated Using Thermo-Mechanical Extrusion—Hydrolysis Efficiency, Fermentation Inhibitors, and Ethanol Yield. *Appl. Biochem. Biotechnol.* **2012**, *166*, 576–589. [\[CrossRef\]](#)
132. Yoo, J.; Alavi, S.; Vadlani, P.; Amanor-Boadu, V. Thermo-Mechanical Extrusion Pretreatment for Conversion of Soybean Hulls to Fermentable Sugars. *Bioresour. Technol.* **2011**, *102*, 7583–7590. [\[CrossRef\]](#)
133. Lamsal, B.; Yoo, J.; Brijwani, K.; Alavi, S. Extrusion as a Thermo-Mechanical Pre-Treatment for Lignocellulosic Ethanol. *Biomass Bioenergy* **2010**, *34*, 1703–1710. [\[CrossRef\]](#)
134. Souza, M.F.; Devriendt, N.; Willems, B.; Guisson, R.; Biswas, J.K.; Meers, E. Techno-Economic Feasibility of Extrusion as a Pretreatment Step for Biogas Production from Grass. *BioEnergy Res.* **2022**, *15*, 1232–1239. [\[CrossRef\]](#)
135. Karunanithy, C.; Muthukumarappan, K. A Comparative Study on Torque Requirement during Extrusion Pretreatment of Different Feedstocks. *BioEnergy Res.* **2012**, *5*, 263–276. [\[CrossRef\]](#)
136. Karunanithy, C.; Muthukumarappan, K. Optimizing Extrusion Pretreatment and Big Bluestem Parameters for Enzymatic Hydrolysis to Produce Biofuel Using Response Surface Methodology. *Int. J. Agric. Biol. Eng.* **2011**, *4*, 61–74.
137. Karunanithy, C.; Muthukumarappan, K. Optimization of Alkali Soaking and Extrusion Pretreatment of Prairie Cord Grass for Maximum Sugar Recovery by Enzymatic Hydrolysis. *Biochem. Eng. J.* **2011**, *54*, 71–82. [\[CrossRef\]](#)
138. Karunanithy, C.; Muthukumarappan, K. Influence of Extruder and Feedstock Variables on Torque Requirement during Pretreatment of Different Types of Biomass—A Response Surface Analysis. *Biosyst. Eng.* **2011**, *109*, 37–51. [\[CrossRef\]](#)
139. Weaver, J.C.; Chizmadzhev, Y.A. Theory of Electroporation: A Review. *Bioelectrochem. Bioenerg.* **1996**, *41*, 135–160. [\[CrossRef\]](#)
140. Golberg, A.; Sack, M.; Teissie, J.; Pataro, G.; Pliquet, U.; Saulis, G.; Stefan, T.; Miklavcic, D.; Vorobiev, E.; Frey, W. Energy-Efficient Biomass Processing with Pulsed Electric Fields for Bioeconomy and Sustainable Development. *Biotechnol. Biofuels* **2016**, *9*, 94. [\[CrossRef\]](#)
141. Rocha, C.M.R.; Genisheva, Z.; Ferreira-Santos, P.; Rodrigues, R.; Vicente, A.A.; Teixeira, J.A.; Pereira, R.N. Electric Field-Based Technologies for Valorization of Bioresources. *Bioresour. Technol.* **2018**, *254*, 325–339. [\[CrossRef\]](#)
142. Brahim, M.; Checa Fernandez, B.L.; Regnier, O.; Boussetta, N.; Grimi, N.; Sarazin, C.; Husson, E.; Vorobiev, E.; Brosse, N. Impact of Ultrasounds and High Voltage Electrical Discharges on Physico-Chemical Properties of Rapeseed Straw's Lignin and Pulp. *Bioresour. Technol.* **2017**, *237*, 11–19. [\[CrossRef\]](#)
143. Barba, F.J.; Brianceau, S.; Turk, M.; Boussetta, N.; Vorobiev, E. Effect of Alternative Physical Treatments (Ultrasounds, Pulsed Electric Fields, and High-Voltage Electrical Discharges) on Selective Recovery of Bio-Compounds from Fermented Grape Pomace. *Food Bioprocess Technol.* **2015**, *8*, 1139–1148. [\[CrossRef\]](#)
144. Vorobiev, E.; Lebovka, N. Pulsed Electric Field in Green Processing and Preservation of Food Products. In *Green Food Processing Techniques: Preservation, Transformation and Extraction*; Academic Press: Cambridge, MA, USA, 2019; pp. 403–430. [\[CrossRef\]](#)
145. Kumar, P.; Barrett, D.M.; Delwiche, M.J.; Stroeve, P. Pulsed Electric Field Pretreatment of Switchgrass and Wood Chip Species for Biofuel Production. *Ind. Eng. Chem. Res.* **2011**, *50*, 10996–11001. [\[CrossRef\]](#)
146. Zhang, L.; Wang, L.J.; Jiang, W.; Qian, J.Y. Effect of Pulsed Electric Field on Functional and Structural Properties of Canola Protein by Pretreating Seeds to Elevate Oil Yield. *LWT* **2017**, *84*, 73–81. [\[CrossRef\]](#)
147. Basak, S. The Potential of Pulsed Magnetic Field to Achieve Microbial Inactivation and Enzymatic Stability in Foods: A Concise Critical Review. *Futur. Foods* **2023**, *7*, 100230. [\[CrossRef\]](#)
148. Szwarc, D.; Szwarc, K. Use of a Pulsed Electric Field to Improve the Biogas Potential of Maize Silage. *Energies* **2020**, *14*, 119. [\[CrossRef\]](#)
149. Zhang, H.; Zhang, Z.; He, D.; Li, S.; Xu, Y. Optimization of Enzymatic Hydrolysis of Perilla Meal Protein for Hydrolysate with High Hydrolysis Degree and Antioxidant Activity. *Molecules* **2022**, *27*, 1079. [\[CrossRef\]](#)
150. Liu, Y.; Tang, Y.; Gao, H.; Zhang, W.; Jiang, Y.; Xin, F.; Jiang, M. Challenges and Future Perspectives of Promising Biotechnologies for Lignocellulosic Biorefinery. *Molecules* **2021**, *26*, 5411. [\[CrossRef\]](#)
151. Li, X.; Shi, Y.; Kong, W.; Wei, J.; Song, W.; Wang, S. Improving Enzymatic Hydrolysis of Lignocellulosic Biomass by Bio-Coordinated Physicochemical Pretreatment—A Review. *Energy Rep.* **2022**, *8*, 696–709. [\[CrossRef\]](#)

152. Rodionova, M.V.; Bozieva, A.M.; Zharmukhamedov, S.K.; Leong, Y.K.; Lan, J.C.-W.; Veziroglu, A.; Veziroglu, T.N.; Tomo, T.; Chang, J.S.; Allakhverdiev, S.I. A Comprehensive Review on Lignocellulosic Biomass Biorefinery for Sustainable Biofuel Production. *Int. J. Hydrogen Energy* **2022**, *47*, 1481–1498. [[CrossRef](#)]
153. Lopes, A.M.; Ferreira Filho, E.X.; Moreira, L.R.S. An Update on Enzymatic Cocktails for Lignocellulose Breakdown. *J. Appl. Microbiol.* **2018**, *125*, 632–645. [[CrossRef](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.